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Award Number: DAMD17-00-C-0018

TITLE: Autonomic Dysfunction in Gulf War Veterans

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REPORT DATE: November 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 2004	Final (31 Jan 00 - 31 Oct 04)

4. TITLE AND SUBTITLE
Autonomic Dysfunction in Gulf War Veterans

5. FUNDING NUMBERS
DAMD17-00-C-0018

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

We hypothesized that exaggerated reactivity of the autonomic nervous system (ANS) under stress, wartime exposures and/or butyrylcholinesterase (BChE) mutations might lead military personnel to develop symptoms associated with Gulf War Illnesses (GWI). We performed two studies designed to test these hypotheses. Study 1 examined wartime exposures and related them to BChE genotype in 160 Cases and 144 Controls. Study 2 examined Cases, Deployed Controls and Non-Deployed controls with a comprehensive ANS test battery, BChE genotype and wartime exposures. We found that BChE genotype, *by itself*, does not determine Case/Control status, but this genotype can interact with some wartime exposures (especially pyridostigmine bromide) to greatly increase the risk of GWI. In addition, veterans who met rigorous criteria for GWI showed measurable, objective differences in a number of ANS endpoints when compared to Control groups. In summary, the results of the present studies indicate that in our samples of Gulf War Veterans, GWI was associated with: (1) altered autonomic function, (2) exposure to pyridostigmine bromide, and (3) being carriers of mutations of BChE when combined with exposure to pyridostigmine bromide. This last interaction produced the largest significant risk, which remained elevated when analyses were recomputed using the more lax CDC Case definition.

14. SUBJECT TERMS
Gulf War Illness, pyridostigmine, butyrylcholinesterase, autonomic nervous system, mutations, wartime exposures.

15. NUMBER OF PAGES
131

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Section 1. Introduction

For military personnel, the sequellae of war include the immediate dangers of combat and the potential for long-term medical and psychological disability. Factors such as fatigue, hunger, lack of sleep, and multiple chemical and physical exposures combine to present the soldier with cumulative stresses[1], which might lead to long-term medical problems. Historically, a number of such "war syndromes" have been described [2,3]. The unexplained symptoms and conditions reported by Gulf War Veterans (GWV) have been labeled "Gulf War Syndrome" by media reports [4]. Several review panels have concluded, however, that there does not appear to be a single, unique syndrome associated with Gulf War service, but numerous investigators using different study designs and populations describe a fairly consistent set of symptom types and illness categories that appear to occur at significantly higher rates in different groups of GWV than military personnel serving elsewhere [5,6,7,8,9,10,11,12,13,14,15,16]

We reasoned that exaggerated reactivity of the autonomic nervous system (ANS) under stress, wartime exposures and/or a particular genetic mutation that alters the function of neural pathways using acetylcholine as a transmitter, might lead military personnel to develop long-lasting symptoms such as those associated with Gulf War Illnesses (GWI). During this project we performed two studies designed to test these hypotheses. In Study 1, questionnaire data and blood samples for genetic analyses were collected from veterans who met criteria for GWI, and from veterans without such symptoms. Study 2 tested veterans with GWI, veterans from the same U.S. Army units who did not have such symptoms, and veterans who served in the U.S. Army during the Gulf War, but were not deployed to the Persian Gulf area. To perform these multidisciplinary studies, members of the project team combined their expertise and research facilities in the areas of epidemiology, genetics and molecular biology, psychophysiology, and human physiological testing.

To determine whether alterations in ANS reactivity or genetic mutation is associated with GWI, it is first necessary to have reliable criteria for identifying GWI veterans. The Centers for Disease Control and Prevention (CDC) derived a Case definition for "multisymptom illness" among Gulf-era veterans, and reported good differentiation between deployed and nondeployed veterans[14]. Others have also found the CDC criteria to be useful in distinguishing British veterans who served in the Gulf War from nondeployed era veterans or veterans who served in Bosnia. Dr. Lea Steele, a member of this project team, directed the Kansas Persian Gulf War Veterans Health Project (KVP), which included an epidemiologic survey of 2,031 Gulf War-era veterans residing in Kansas[17]. The prevalence of CDC-defined multisymptom illness was 47% in Kansas GWV, compared to 20% among nondeployed era veterans. In the course of her study, Dr. Steele identified a number of highly correlated symptom groupings, which individually and collectively, occur at significantly higher rates among deployed than nondeployed veterans. This information was used to derive a more rigorous Case definition of GWI (e.g., in the KVP Case definition, symptoms are counted only if they first began in 1990 or later, and persisted or recurred over the preceding year). We chose to use the new KVP criteria in the current studies because they provide better differentiation between Cases and Controls when compared with the CDC criteria. Dr. Steele's participation in this project allowed us to examine various associations between the basic epidemiological parameters of GWI and laboratory-derived measures of human physiology and genetics.

Dr. Oksana Lockridge of the Eppley Institute, University of Nebraska Medical Center at Omaha is also a member of this project team. Based on some of her earlier findings[18], we hypothesized that there would be a strong correlation between ANS symptomatology and being a heterozygous carrier of the A or F variant of the enzyme butyrylcholinesterase [BChE; EC 3.1.1.8]. She found such carriers to be present in a much greater proportion (9:1-10:1) in self-selected veterans with self-reported symptoms of GWI than in veterans without such symptoms. There was also a much weaker association with homozygous carriers for the K mutation. Dr. Lockridge's participation allowed us to attempt to replicate these findings, and to further examine the role genetics plays in GWI, by applying the methods of molecular biology to a large, well-defined population of GWV and matching Controls.

The activity and feedback loops of the ANS are too varied and complex to be captured in a single test. For this reason it is routine to examine ANS activity using a battery of tests, each designed to modify the activity of the ANS in a different way. Many of these tests challenge the individual with physical, cognitive or emotional stressors (e.g., Valsalva maneuver, mental arithmetic, recall of stressful events), and analysis of the effects of such tests involves the complex mathematical quantification of physiological activity according to different indices or metrics. For example, a complete description of the Valsalva maneuver includes the Valsalva ratio, the heart rate overshoot latency, and several other cardiac and blood pressure measurements. To determine whether veterans with symptoms of GWI differ in autonomic reactivity from veterans without such symptoms, the co-principal investigator (Dr. Mary Cook), with the assistance of the principal investigator (Dr. Sastre), designed a battery of tests known to affect the ANS in different ways. The battery included eight challenges, with continuous recording of the electrocardiogram (ECG), non-invasive tonometric (continuous waveform) blood pressure and respiration to examine ANS reactivity. The study design allowed for examination of baseline group differences between Cases and deployed and nondeployed Controls, differences in reactivity to stressors in these three groups, and any effects that BChE genotype may have on ANS baseline or reactivity to stressors. Dr. Sastre had previously developed efficient and reliable methods for the extraction and mathematical analysis of complex continuous physiological waveforms; without such methods, the physiological study reported here would not have been feasible.

From the inception of our study, our position was not that GWI is a single syndrome, but that with more refined analyses, veterans reporting GWI symptoms can be classified into a small group of well defined symptom clusters, one of which will prove to have an autonomic etiology with prominent autonomic symptoms. We also hypothesized that alterations in the function of central and peripheral neural pathways that use acetylcholine as a transmitter are important elements in ANS dysfunction. Further, we believed that dysfunction in cholinergic metabolic pathways (including tissue and circulating BChE) can lead to functional alterations in end-organ responses.

Towards the end of the originally-scheduled completion date of the starting scope of work of our study (January, 2003), it became evident that our data required more extensive analyses than had been foreseen in the originally-approved scope of work. In addition, concurrent investigations by other research groups in the U.S. and in the U.K. had suggested, in preliminary studies, that different genotypes or velocities of the enzyme Serum Paraoxonase 1 [PON1, EC 3.1.8.1] may be a risk factor for development of GWI. For these reasons, the original scope of work was expanded, and the completion date extended so that further analyses of the data could be performed. In addition, veterans were re-contacted and their permission obtained to use previously-collected blood samples for determination of PON1 genotype and enzyme velocity with three different substrates. This was done in order to ascertain

if the preliminary results reported elsewhere on a possible association between PON1 genotypes or velocities and risk of development of GWI were corroborated in our sample of veterans.

Section 2.
Body

2.1 Methods and Experimental Design

2.1.1 Study 1.

Experimental Design and Volunteers

The study compared two groups of veterans: approximately 150 Gulf War veterans who meet the CDC criteria for GWI and approximately 150 who were deployed to the Gulf and who do not meet symptom criteria for GWI. Study volunteers were recruited from individuals included in the KVP database who live in the Kansas City area. Study volunteers were also recruited, using advertisements, from individuals not included in the KVP database who live in the Kansas City area. All veterans served in one of the U.S. Armed Forces, and approximately 10% were women. The study protocol was reviewed and approved by the MRI Institutional Review Board (IRB) and the Surgeon General of the Army's Human Volunteers Research Review Board (HSRRB). Written informed consent was obtained from all volunteers participating in the study.

Inclusion and Exclusion Criteria

The volunteers for Study 1 were required to speak, read and write English, and meet the additional inclusion and exclusion criteria associated with being in the GWI or Healthy group. Criteria for excluding volunteers were the same as those used by the KVP: cancer (other than skin cancer, excepting melanoma), diabetes, heart disease (other than high blood pressure), stroke, multiple sclerosis, lupus, long-term problems from serious injuries, chronic infections lasting over 6 months (e.g., tuberculosis, hepatitis, HIV), history of serious psychiatric disorders (schizophrenia, bipolar disorder) or any current psychiatric disorder that required hospitalization since 1991 (depression, PTSD, alcoholism, drug dependence).

Volunteer Recruitment and Data Collection

Volunteer recruitment for study 1 began on September 15, 2000, and was completed on December 6, 2000. Volunteer appointments started on September 25, 2000, and were completed on December 20, 2000. Data collection for study 1 was anticipated to require 12 months but was completed in 4 months, ahead of schedule, thanks to the enthusiastic participation of the volunteers who were contacted. Each veteran completed a questionnaire to provide information on current symptoms as well as service-related exposures and military history, and provided a blood sample for BChE analysis.

Common Study 1 & 2 Biochemistry and Molecular Biology Procedures

Blood Collection

Two 9.5 mL tubes of blood were collected from each volunteer. The first tube contained no anticoagulant. After clotting, the blood was centrifuged, and the serum supernatant was stored at ~ -20°C until assayed for BChE phenotype.[25] The second tube contained citrate anticoagulant. The tube was spun down and the buffy coat harvested. The buffy coat was stored ~ –20°C until ready for assay as source of DNA for genotyping the F and K variants. After the removal of the buffy coat, the tube containing the packed red blood cells was vortexed briefly to resuspend the cells. The cells were mixed 1:1 with a citrate-phosphate buffer, pH 6.0, and stored at ~ –20°C.

Enzyme Activity

For phenotyping, enzyme activity was measured with 50 μM benzoylcholine as the substrate[19] in 0.067 M Na/K phosphate buffer, pH=7.4 at 25°C. Hydrolysis was measured spectrophotometrically at 240 nm and activity calculated from $\Delta E = 6.7\ mM^{-1}cm^{-1}$ and expressed as micromoles benzoylcholine hydrolyzed per min per mL of serum, defined as units per mL (U/mL) at 25°C. Inhibition of activity by 10 μM dibucaine was used to identify the "atypical" and fluoride-resistant phenotypes. In Cases of unusual dibucaine inhibition, degree of inhibition obtained with 50 μM NaF was measured to distinguish between the UA, UF, AF, FF, and FS phenotypes.[25,20,21]

DNA Preparation and Analysis

DNA was isolated from the buffy coat layer using the IsoCode PCR DNA Sample Isolation Device (Schleicher & Schuell); established procedures to reduce possible contamination of DNA samples by other DNA were used. A one-eighth-inch punch was used to punch out dozens of filter circles from a single IsoCode paper strip. Thawed buffy coat or leukocytes, about 5 μL, were applied to each filter circle. Several filter circles of the same sample are placed inside a closed microtube containing Drierite, and the tube covered with a KimWipe plug. Filter circles were dried overnight at 37°C, and rinsed in 500 μL distilled autoclaved water with 5 sec pulse-vortexing. To elute genomic DNA, one filter circle of blood was placed in a 0.5-mL tube containing 50 μL distilled autoclaved water. The tube was heated at 95°C for 30 min, pulse-vortexed 15 times after 15 min, and then 60 times after 30 min. PCR amplification of genomic DNA followed by restriction enzyme digestion was used to genotype DNA at the polymorphic site for BChE located at Ala/Thr 539. Wild-type BChE has Ala 539, whereas the K-variant has Thr 539 in this position[22,23]. PCR reactions consisted of 3 to 7 μL of genomic DNA in a 50-μL reaction. Taq polymerase (Promega) and 3 mM $MgCl_2$ were used in the reaction. The annealing temperature was 57°C to 60°C. Four primers for two different PCR amplifications have been designed and were used.[25] The A amplification creates a Mae III restriction site when the K-variant ACA codon (Thr 539) is present. The B amplification creates a Bgl I restriction site when the GCA codon (Ala 539) is present. Because of previous disappointing work using a primer that created a Dra I site, the more expensive but more reliable Mae III (Roche Molecular Biochemicals) was used along with a new amplification primer that created a Mae III site in K-variant alleles. Mae III has been used to detect the K-variant mutation.[25,24,25]

DNA of samples that phenotype as heterozygous for the F variant of BChE were amplified and sequenced to determine which of the three reported DNA mutations are responsible for fluoride resistance.[26,27] It was not necessary to genotype samples that phenotype as heterozygous for the BChE A variant[28] (Asp 70 to Gly) because dibucaine inhibition of serum activity was extremely accurate in this determination.

Carbamate Affinity Testing

We measured carbamate affinities to AChE with a radioisotopic assay based upon the quantitation of [^{3}H]acetate produced by hydrolysis of labeled [^{3}H]acetylcholine, as described by Johnson and Russell (1975),[29] modified by Nostrandt et al. (1993),[30] and further modified in our lab to increase the extraction efficiency of the ^{3}H-labeled acetate into the fluor and reduce sample variation. This assay permitted the use of essentially undiluted samples. Incubation with carbamates and no substrate for one hour achieved a plasma-like equilibrium. Total assay time after addition of substrate was no more than 30 sec. Our standard substrate was unlabelled acetylcholine iodide (15 mM) with tracer [acetyl-H^3] acetylcholine iodide (0.23 mM). Assays were run in triplicate for each specimen, and a substrate blank was run in duplicate at least every hour once the incubations began to determine the amount of spontaneous hydrolysis of the acetylcholine. Samples were incubated with three concentrations of pyridostigmine bromide (PB) between 0.1 and 1 μM for 1 hour before residual activity was assayed; samples with K_{app} outside our population ranges were reassayed with seven concentrations between 0.05 and 10 μM. Our internal control was a commercially available compound containing AChE and BChE at known levels.

2.1.2 Study 2.

Experimental Design and Volunteers

Our protocol called for three groups of enlisted Army veterans: Cases, Deployed Controls (DC), Nondeployed Controls (NDC), and one group consisting of at least 20 veterans identified as heterozygous for atypical (A) or fluoride (F) variants, or homozygous for the K mutation of BChE in Study 1. Volunteers were drawn from the KVP. The planned strategy was to select Cases and DC from the same units in order to help control for exposure to chemicals, smoke, and other environmental factors that were present in the Gulf War. Volunteers were drawn from more than one unit to increase the generalizability of the results.

The KVP epidemiologic study found that rates of GWI varied as a function of branch of service, rank, sex, and deployment location. Therefore, the Case/Control sampling frame for Study 2 was designed to include Case and Control volunteers who were similar in terms of those key parameters. All individuals in the sampling frame served as enlisted personnel in the U.S. Army between August 2, 1990, and July 31, 1991. Study protocols were reviewed and approved by the MRI Institutional Review Board (IRB) and the Surgeon General of the Army's Human Volunteers Research Review Board (HSRRB). Written informed consent was obtained from all volunteers participating in the study.

Veterans contacted for the study were selected from among eligible veterans in the KVP database who lived in either the Kansas City or Junction City, Kansas, areas. Cases (planned N = 40) and DC (planned N = 25) were selected from among veterans who had been attached to either the 1st Infantry Division or the 410th Army Evacuation Hospital Reserve Unit during the Gulf War. NDC (planned N =

25) were selected from among Army enlisted personnel who served with any unit during the time of the war, but had never deployed to the Persian Gulf area. A stratified random sample was selected from among those in the sampling frame, to provide an equal proportion of women (approximately 10%) among Cases and both Control groups.

Telephone numbers for all volunteers in the sampling pool were reviewed and updated, when possible, using Internet files and telephone directories. Prior to making telephone contact, potential volunteers were sent letters describing the study and requesting that they call the study hotline if the phone number listed for them was incorrect. After informing potential volunteers about the study and verifying that they met military service inclusion criteria, interviews were requested of eligible veterans. Interviews were conducted by one of two trained interviewers using a CATI (computer assisted telephone interviewing) system. The screening interview asked veterans about medical and psychiatric conditions for which they had been diagnosed by a physician, in order to identify those who were medically ineligible for the study. Veterans were also asked about persistent symptoms experienced since the war in order to assign each with a provisional designation of Case or Control, using the KVP Gulf War illness Case definition described previously.

The other study group included in Study 2 consisted of homozygote carriers of the K mutation or heterozygote carriers of the A and F mutations of BChE (Variants, planned N at least 20). All 28 heterozygotes identified in Study 1 were contacted and invited to participate. This group included both enlisted personnel and officers, and those who served in any service branch or military unit, with no stratification by sex.

Inclusion and Exclusion Criteria

Exclusion criteria for Study 2 were the same as those for Study 1. Volunteers were required to speak, read and write English, and meet the additional inclusion and exclusion criteria associated with being in the Case or Control groups. Veterans were medically excluded if they reported ever being diagnosed with cancer (other than skin cancer, excepting melanoma), diabetes, heart disease (other than high blood pressure), stroke, multiple sclerosis, lupus, chronic infections lasting over 6 months (e.g., tuberculosis, hepatitis, HIV), history of serious psychiatric disorders (schizophrenia, bipolar disorder) or any psychiatric disorder that required hospitalization since 1991 (depression, PTSD, alcoholism, drug dependence). Female veterans were excluded if they reported being pregnant.

Volunteer Recruitment and Data Collection

After completing the screening interview, veterans found to be eligible for the study were informed about the nature of the study and physiologic testing, and invited to schedule an appointment at the testing site. Veterans who agreed to participate were mailed an information package, which included the study questionnaire. Veterans were asked to bring their completed questionnaire with them to the testing site when they came in for their appointments. They were also instructed to consume caffeine and tobacco in their typical manner, to have a light meal before arriving at the testing site, but to refrain from drinking alcoholic beverages the night before testing.

Upon arrival at the testing site, the study was again described, including a detailed description of testing procedures, and written informed consent was obtained. Veterans also completed the Volunteer

Registry data sheet. The investigator reviewed the written questionnaire for completeness, and clarified any confusing or missing answers with the veteran. Some volunteers forgot the packet or forgot to fill it out. Such individuals were given any needed materials and asked to finish a packet before testing began.

Testing was conducted at MRI and at Junction City, Kansas. Junction City was selected because the area has a large concentration of veterans; this optimized convenience for the volunteers. When data collection had ended, each volunteer had: (1) completed an autonomic reactivity battery (ATB) designed to evaluate responses to a variety of stimuli and situations that affect the ANS; (2) provided blood samples for genetic analysis; and (3) completed a set of questionnaires selected to provide information on symptoms, military service, and personality factors that might affect autonomic function.

Questionnaires

Volunteers were asked to provide information on their current symptoms, as well as service-related exposures and military history. The answers were used to confirm that the volunteer met criteria for participation, and for subsequent examination of exposure and symptom patterns. The questionnaire included items from the Autonomic Symptom Profile developed at the Mayo Clinic;[31] and the veteran's version of the SF-36,[32,33] a general symptom checklist with demonstrated reliability.

Physiological Recording

Tilt testing methods were similar to those used at the Mayo Clinic and described in the consensus document from the AAS/AAN for definition of various disorders involving syncope.[34,55] Methods for other tasks followed traditional psychophysiological and autonomic evaluation procedures as described below for each task. The electrocardiogram (ECG), tonometric blood pressure (BP) from the dominant arm, and respiration were measured continuously throughout all the tests in the battery except during the pre-pulse inhibition task. Data were sampled at 256 Hz and stored in magnetic media for off-line data processing. Electromyographic (EMG) measures from the orbicularis oculi, sampled at 1024 Hz, provided quantitative information for analysis of the amplitude of the startle reflex and its inhibition (PPI).

ECG

The ECG was recorded using disposable "snap" electrodes applied to prepared skin sites on the right and left clavicles and the seventh intercostal space under the left ancillary midline, corresponding to the standard ECG Lead II configuration. ECG activity was recorded using Grass Neurodata Model 15 (Grass Instrument Division, Astro-Med, Inc., Warwick, Rhode Island) multi-channel physiological recording equipment and sampled at 256 Hz for determination of mean heart rate (MHR) and heart rate variability (HRV) from R-R intervals. Our laboratory has developed and validated custom software[35] for automatic detection of R-wave fiducial points, assessment of R-R intervals and computation of time-domain and spectral HRV parameters using the U.S.–European Consensus Guidelines.[36]

The spectral HRV parameters that were computed included total power (Power), absolute and relative low-frequency power (ABS LF and %LF; these are believed to be, in part, reflective of sympathetic activity), absolute and relative high-frequency power (ABS HF and %HF; these are

primarily a reflection of parasympathetic activity), and low-frequency power to high-frequency power ratio (L/H). Typically we limited spectral analyses to recording periods of 3 minutes or longer. Others [37] have found that 5-min recording periods for measurement of HRV were consistent over time, and Sloan et al.[38] found significant correlations between HRV obtained from 5-min recordings compared to 24-hour recordings. While intervals as short as 2.5 min have been used,[39] in our experience such short periods produce unacceptably noisy data.

Time domain measures included SDNN, the standard deviation of normal interbeat intervals (IBI), rMSSD, the square root of the mean squared differences of successive IBI, NN50, the number of interval differences of successive IBI greater than 50 ms, and pNN50, the proportion derived by dividing NN50 by the total number of IBI in a given recording period. In a strict mathematical sense, total spectral power (from 0.0 Hz to the Nyquist sampling limit of 0.5 Hz) is identical to SDNN. However, the U.S.–European Consensus Guidelines recommend that total spectral power be reported for frequencies ≤ 0.4 Hz, therefore SDNN and total spectral power, when computed according to these guidelines, are not redundant measures.

Tonometric Measures of BP

In studies of autonomic reactivity, reproducibility over time is limited by the fact that relatively few measures of BP can be obtained using automatic auscultation methods. Tonometric techniques[40,41] allow the continuous noninvasive measurement of the full BP waveform from the radial artery. The instrument we used (Colin Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas) has FDA approval. This device has an array of sensors that flatten the arterial wall, and software/hardware to optimize the pressure of the sensor and the specific sensor position in the array that provides the measurement. This approach makes it much easier to position the sensor appropriately over the wrist, a technique that is very difficult when only one sensor is used. Kemmotsu et al[44,45] have reported correlations between tonometric and invasive intra-arterial BPs of 0.94 to 0.97 during anesthesia. Weiss et al.[42] reported lower correlations; however, they conclude that tonometric measurements provide a reliable indicator of changes in pressure during induction of anesthesia, and can be appropriate when arterial cannulation is not feasible.

Movement artifact presents the greatest challenge to accurate tonometric measures of BP. Our experience during development of the protocol and testing of the procedures indicated that this source of artifact is reduced by splinting the hand and wrist on which the sensor array is placed, and by maintaining the arm at heart level. Nonetheless, this part of the protocol was still the one most prone to having missing data. In addition to movement artifacts that were clearly identifiable, in a small subpopulation of volunteers there was also drift in the hold-down pressure for the tonometric sensor. When the drift happened during the tilt-up procedure, the data obtained in this subset of volunteers was of questionable validity. This was less of a problem for the tonometric data in other parts of the protocol, because if hold-down pressure drift occurred, the sensor was reset and a new control period of data obtained.

The calibrated tonometric BP signal was sampled at 256 Hz. Mean BP was computed by averaging all of the digitized readings over the time duration of a task. Custom software identified the peaks and troughs of the tonometric BP signal as the systolic and diastolic pressures on a beat-to-beat basis, and for any given task the systolic pressure (SBP) endpoint reflect the average of the systolic pressure values

obtained during the task. A similar procedure was followed to obtain the diastolic pressure (DBP) endpoint.

Respiration

Respiration was recorded using a Grass Model F-RCT Piezo Trace transducer placed around the chest under the arms or at the level of the diaphragm. As the chest expands and contracts, the piezoelectric sensor is deformed, and the deformation generates a voltage signal proportional to the changes. While under arm placement is optimal for most individuals, records from those who primarily breathe abdominally are difficult to interpret. For such individuals, the gauge is placed on a level with the xyphoid process. The gauge length is adjustable to provide optimal stretch for each individual. Data is recorded using a Grass Neurodata Model 15, with the low filter set to 0.01 Hz. This time constant provides minimally attenuated waveforms, and the data obtained are adequate to describe the rate and relative amplitude of the respiratory cycle.

EMG

The startle response was measured using small biopotential electrodes attached with double-sided adhesive to the orbicularis oculi muscle under the left eye. The site was cleansed first with an alcohol pad, and Grass electrode cream served as the contact medium. The EMG was sampled at 1024 Hz.

2.1.3 Autonomic Testing Procedures

After questionnaires were completed, sensors were attached to measure ECG, BP, and respiration. A normal part of preparing and instrumenting veterans for the physiologic tests included recording BP by auscultation in both arms. Any volunteer who exhibited a SBP of less than 90 mm Hg or a DBP of less than 50 mm Hg in either arm had the BP taken again. If either of the exclusionary readings listed above were present in this second recording, the volunteer participated in all the other phases of the study, lying supine in the tilt table, but the investigators skipped the phase in which the volunteer is tilted head-up. The volunteer then lay down on a standard tilt table (Colin model CM6121.TB, Colin Medical Instruments Corp, San Antonio Texas). Data were collected sequentially using the following procedures; the estimated times listed include procedures, data collection, and answering simple questions:

Resting baseline (5 min): The volunteer remained quietly on the tilt table with no instructions other than to relax for about 5 min while the investigators made sure all the equipment was recording properly. During this time, the resting respiration rate was determined. The volunteer then engaged in paced breathing. The computer was set at the determined rate, and indicated to the volunteer when to inhale. If the volunteer felt that the breathing rate was too fast or too slow, the computer was adjusted and the process continued. Figure 1 illustrates some representative data obtained with our experimental set-up during baseline conditions, prior to engaging the volunteer in paced breathing.

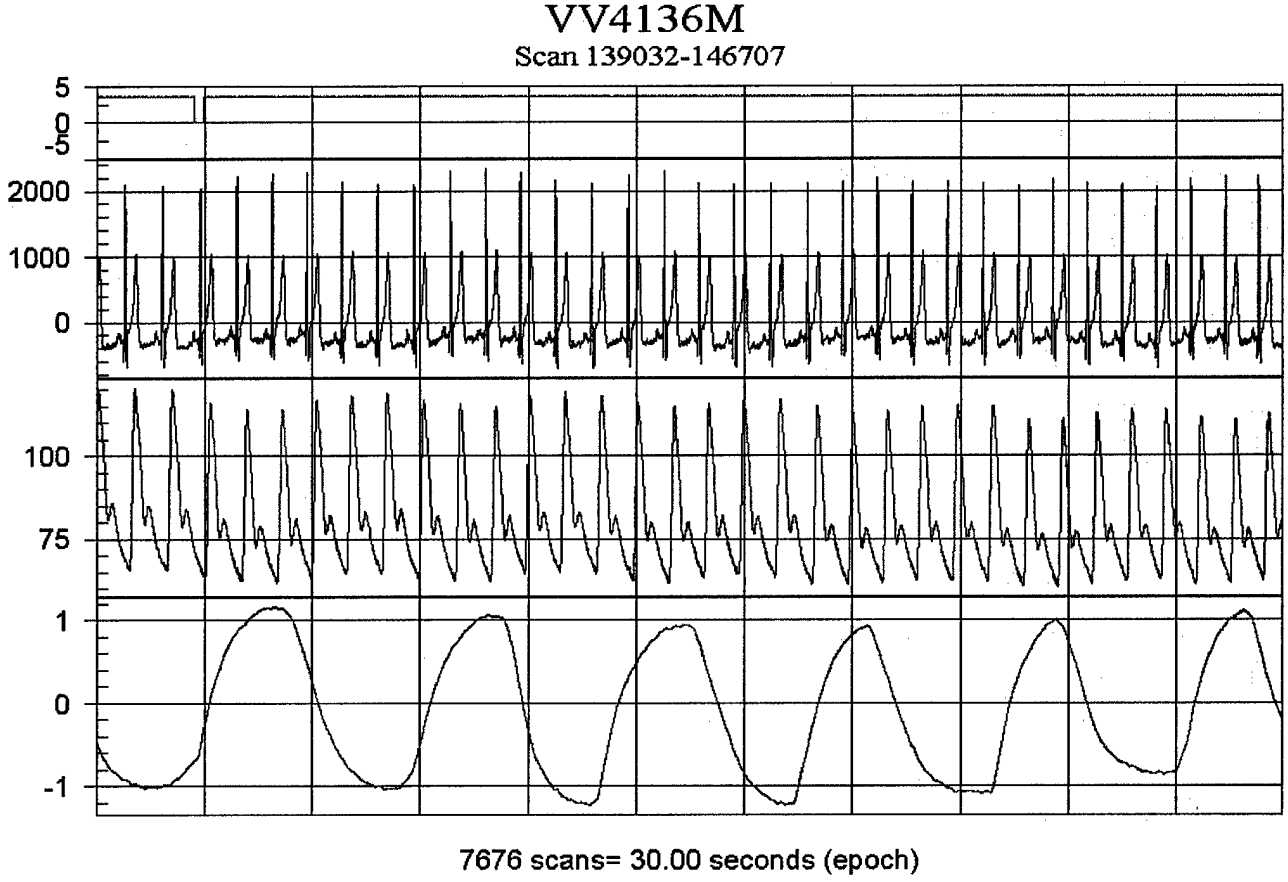


Figure 1 Representative 30-second record of ECG, radial artery tonometric BP and respiration during the first 5-minute baseline period. The screen code VV4136M is a unique identifier for data from this volunteer: Study VV, volunteer #4136, multichannel data sampled at 256 Hz. The upper scan number ranges identify these data from the beginning of the record. The vertical axes for the first (marker), second (ECG) and fourth (respiration) channels are in arbitrary units, the second channel (BP) is in mmHg. The marker channel, shown in green at the top of the figure, indicates the onset of a timed 5-minute baseline period as marked by the investigator.

Deep breathing (6 min): Procedures are similar to those used at the Autonomic Reflex Laboratory at the Mayo Clinic[43,44,45,46,47,48]. The rate of breathing has a profound effect on the high-frequency component of HRV; variability is maximal at 5 to 6 breaths per minute.[49] In our version of the task, inspiration at 33% of the baseline rate determined during paced breathing was signaled for the volunteer by a tone presented over a speaker and by a visual signal. Practice was given to assure that the volunteer understood how to breathe slowly, smoothly, and deeply. After a 1-min rest period, the volunteer performed the deep breathing task for 8 cycles two times, separated by a 1-min rest period.

Sustained hand-grip at 30% of maximum (4 min): The cardiovascular response to a sustained hand-grip consists of an early HR increase due to vagal withdrawal, followed by another increase, presumably due to sympathetic activation. Ewing et al.[50] recommend sustained hand-grip of 30% of maximum for up to 5 min. Low and colleagues[47,48] note that 3 min seems to be adequate, and may be preferable since

many people are unable to maintain the hand-grip for 5 min. A hand dynamometer (Lafayette Instruments Model 76618, Lafayette, Indiana) was used to determine maximum grip strength in the dominant hand. The dynamometer was modified so that grip pressure was presented on a computer. The program determined 30% of the maximal grip. The volunteer was instructed to squeeze the dynamometer to the selected level; then increase grip strength if a tone generated by the computer went lower or decrease it if the tone went higher. Grip strength was sampled at 256 Hz and the average grip strength during each 30 seconds of the task recorded. When the task was completed, the volunteer rated his/her perceived exertion using the Perceived Exertion Scale.[51] Mean HR and BP values were used as outcome measures.

Valsalva maneuver (5 min): In evaluating the response to the Valsalva maneuver it is necessary to analyze both HR and BP response, as the HR response is typically secondary to the change in BP.[52] A modification of the method described by Denq et al.[57] was used. Volunteers were taught to blow into the tube of a dial-type sphygmomanometer with a large face, and to maintain pressure at approximately 40 mm Hg for 15 sec. After a brief rest, the maneuver was repeated until two similar recordings of HR and BP were obtained. The maximal HR generated by the Valsalva maneuver, divided by the lowest HR occurring within 30 sec of the beginning of the test (the Valsalva ratio), provided one outcome measure. This measure takes into account both the early part of Phase II and Phase IV[52,53] of the maneuver. Blood pressure data obtained from the same time points was used to evaluate the primary BP response. Mean HR, mean BP, SBP and DBP during the task were also compared to baseline. Figure 2 illustrates some representative data obtained with our experimental set-up during a Valsalva maneuver.

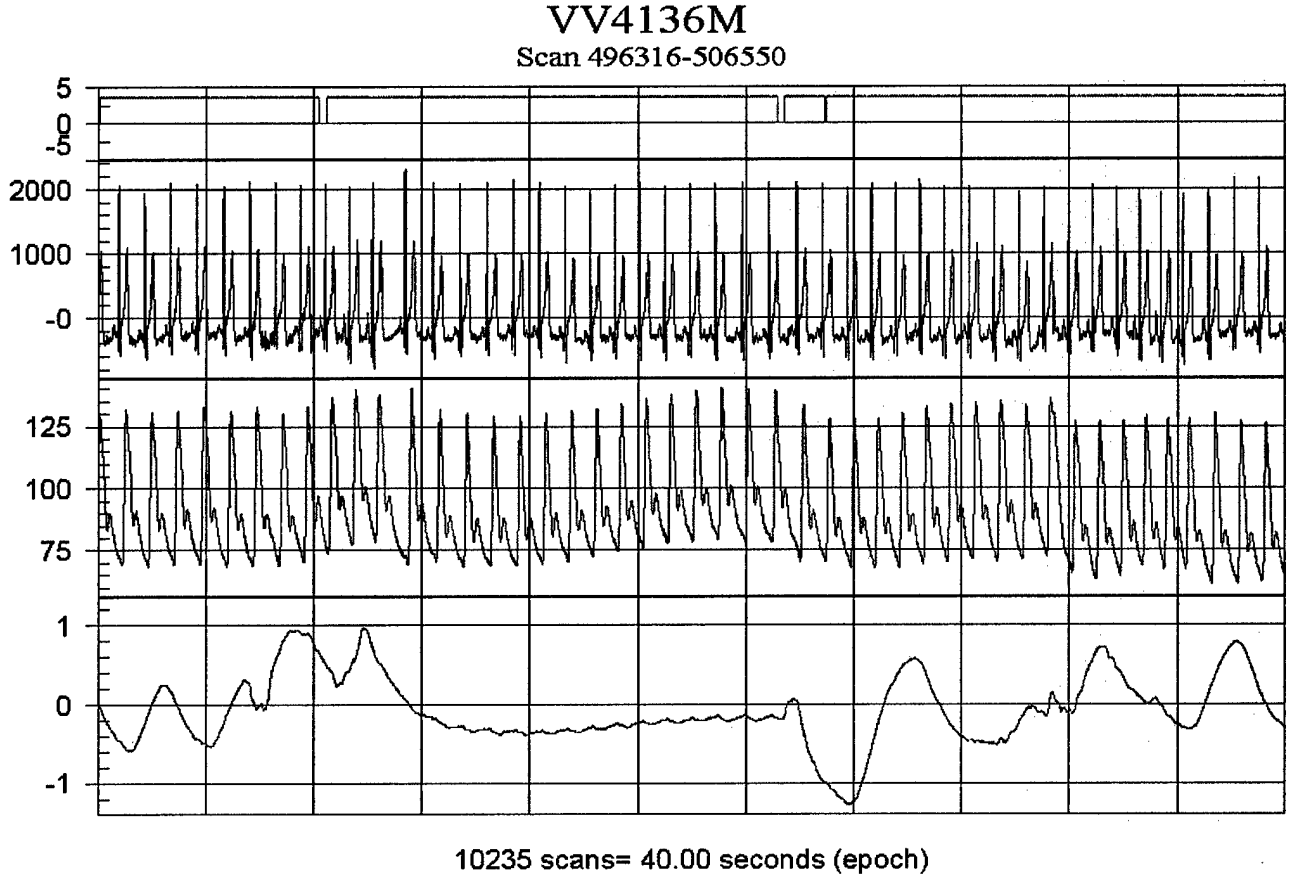


Figure 2. Representative 40-second record of ECG, radial artery tonometric BP and respiration that encompasses a 15-second Valsalva maneuver. The vertical axes are as defined in Figure 1. The marker channel (wide marks) indicates the onset and termination of a timed 15-second Valsalva maneuver as marked by the investigator. The respiration channel shows the volunteer taking one last breath before blowing into the tube, and quickly releasing his breath upon instruction from the investigator.

Quiet rest: The volunteer then rested quietly for 3 min. The last 2 min of this rest period was used as a baseline for the next task (mental arithmetic).

Mental arithmetic (3 min): The volunteer was instructed to sequentially subtract 7s out loud starting from the number 692. To be sure the instructions were understood, a 15-sec practice period using subtraction from a two-digit number preceded the test. Subtraction continued for 2 min. Mean HR, standard deviation of HR, and change in SBP and DBP served as the dependent variables. Figure 3 illustrates some representative data obtained with our experimental set-up during performance of this task.

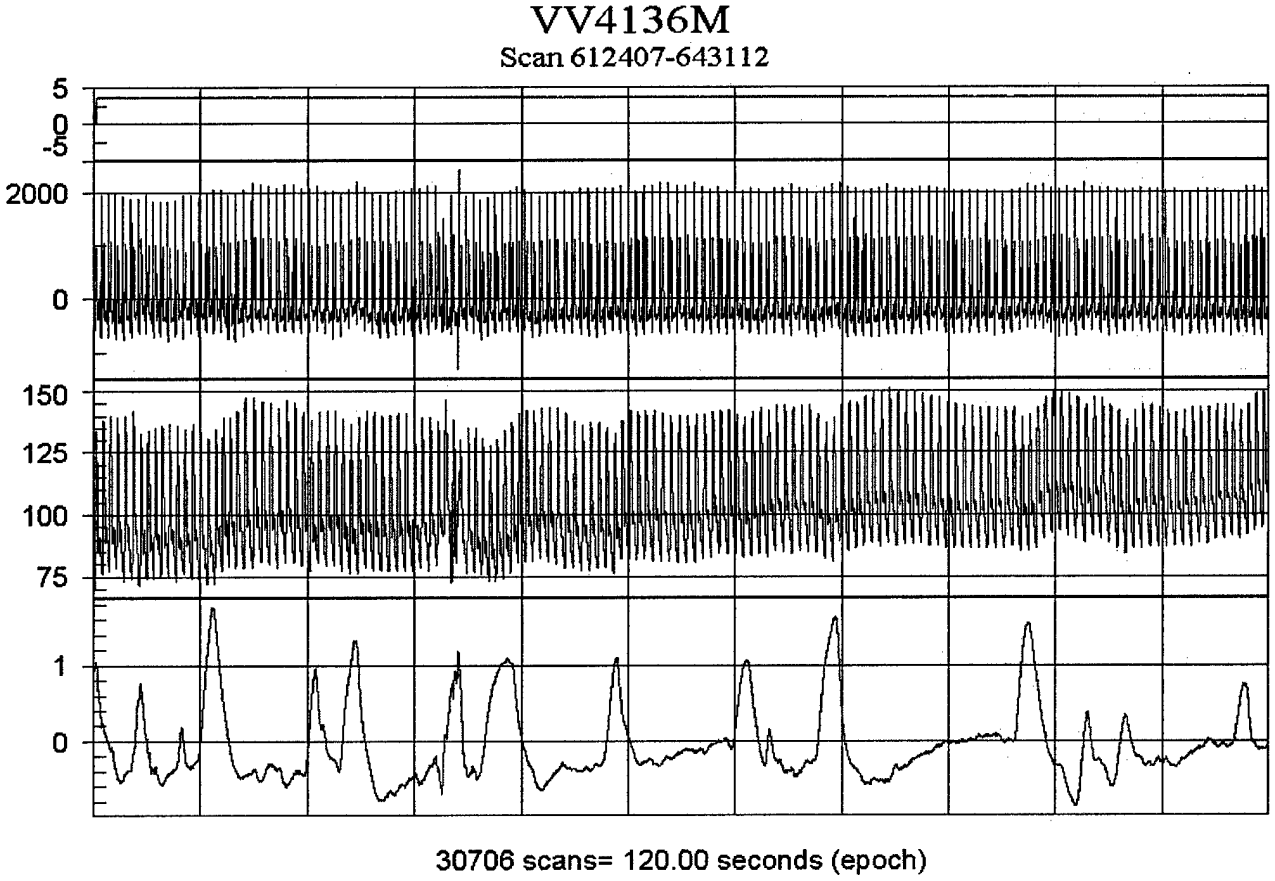


Figure 3. Representative 120-second record of ECG, radial artery tonometric BP and respiration at the beginning of the mental arithmetic task. The vertical axes are as defined in Figure 1. The respiration channel shows the irregular pattern that results as the volunteer articulates the answers. A steady increase in systolic and diastolic BPs is evident as the volunteer progresses with the task.

Quiet rest: The volunteer then rested quietly for 5 min to provide an adequate baseline for the emotional stress task described next.

Emotional Stress (8 min): The volunteer was instructed to spend 90 seconds thinking about "a stressful experience you have had in your life," and to spend 2 to 5 min telling the investigator about it (e.g., where you were, what the environment was like, what happened, how you felt about it). Outcome measures included changes in mean HR, spectral and time-domain measures of HRV, mean SBP, and mean DBP from the baseline period to the exposition period. This task is similar to one used by Cohen et al.[54] in a study of patients with Post-Traumatic Stress Disorder.

Quiet rest: The volunteer then rested quietly for 5 min. Data from this period were used as baseline for the tilt task.

Head-up tilt (20 min): There is an extensive literature on the use of head-up tilt to diagnose autonomic dysfunction. The procedures we use followed the Mayo Clinic[43,44,45,46,47,48] and the consensus statements prepared by the AAS/AAN.[37,55] Our tilt table test, however, was a short research test and not a clinical procedure. The standard clinical tilt table protocols typically last 30-40 min, and if large blood pressure drops, dizziness or fainting are not elicited, infusions of isoproterenol or a small dose of nitroprusside may be used to provide a further cardiovascular stress. The clinical test is not terminated prematurely just by SBP drops unaccompanied by reports of dizziness or fainting. In contrast, our research test used tilt to examine cardiovascular responses during the first 20 minutes, with built-in premature termination criteria designed to make it extremely unlikely that any volunteer would experience fainting or dizziness. In addition, as noted in the methods, we recorded SBP and DPB by non-invasive arterial tonometry, in which the BP values are displayed on the screen on a beat-by-beat basis. Thus there is very little lag time between any significant drop in BP and the investigator being able to restore the volunteer to a supine position. While the potential side effects of the test were noted to the IRB and HSRRB, and are mentioned in the informed consent form, our procedures were designed to extract the maximum amount of research information with the absolute minimum risk of discomfort to the volunteer.

Any volunteer who exhibited a SBP of less than 90 mm Hg or a DBP of less than 50 mm Hg by auscultation in both arms prior to testing did not participate in the head-up tilt task. The volunteers were supine for at least 20 min prior to testing. Since the tests described above take at least 35 min, this requirement was fulfilled. The activities in the preceding portions of the battery were all carried out with the volunteer supine and should not affect the response to tilt. The tilt table, with the volunteer's arm supported at heart level on an arm board and feet resting comfortably on a foot support, was raised (8-9 sec) to 80 degrees, and maintained at that angle for either 20 min or until: the volunteer reported feeling faint; SBP exhibited a sustained drop of more than 30 mm Hg; or DBP exhibited a sustained drop of more than 15 mm Hg, whichever occurred first. If any of these occurred, the table was immediately returned to the horizontal position. Both the initial response to tilt that occurs within the first minute and the sustained response were analyzed using HR, HRV, SBP, and DBP as the outcome variables. Figure 4 illustrates some representative data obtained with our experimental set-up during the response to the first 30 seconds of upright tilt.

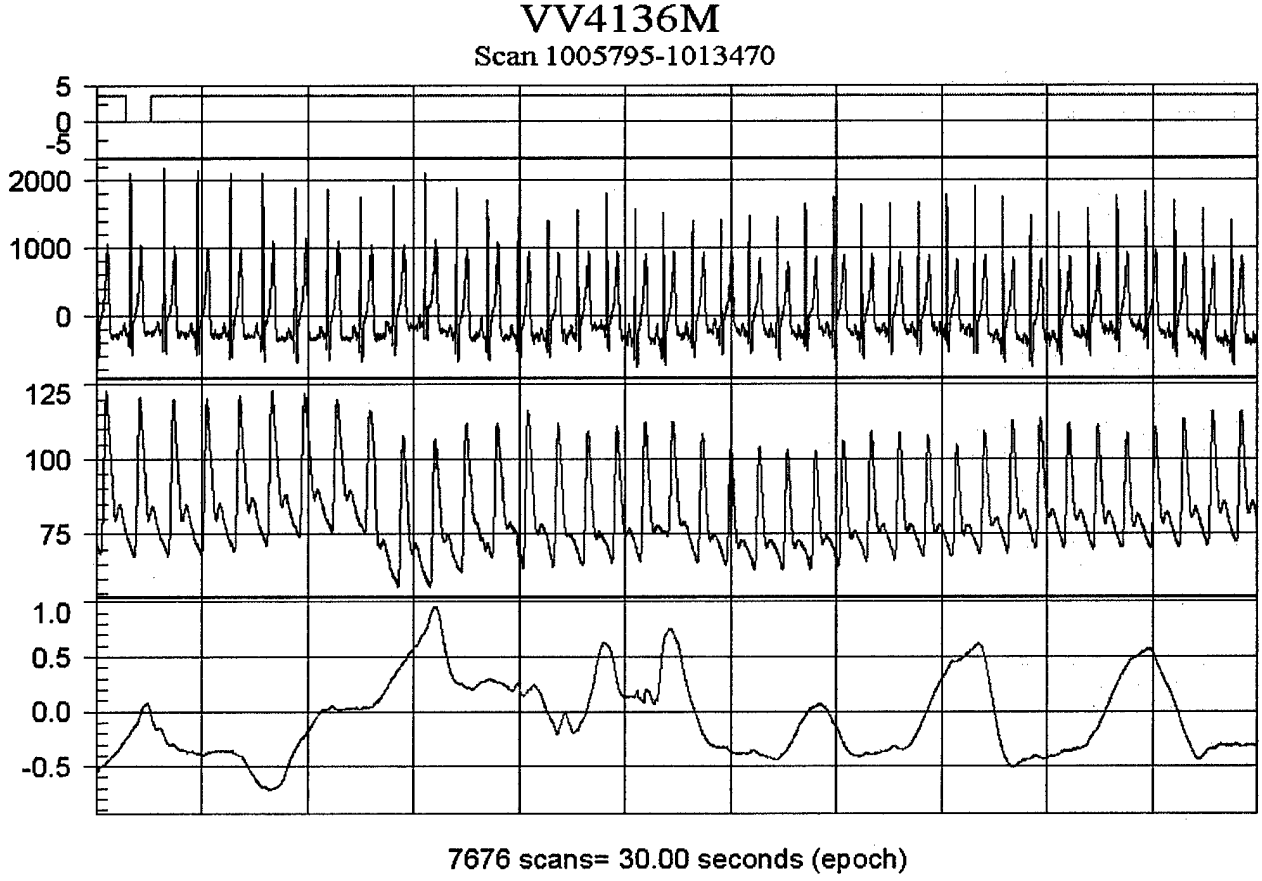


Figure 4. Representative 30-second record of ECG, radial artery tonometric BP and respiration that contains the initial reaction to head-up tilt. The vertical axes are as defined in Figure 1. The marker channel (wide mark) indicates the onset of the upward motion of the tilt table, which takes 8-9 seconds to reach 80 degrees. The BP channel shows a transient drop in both systolic and diastolic BPs before compensatory reflexes overcome the orthostatic pull.

Recovery (up to 20 min): The table was returned to the horizontal position over 8-9 seconds, and the volunteer lay quietly on the tilt table while HR and BP were monitored. The test was terminated after 15 min or, if HR and BP had returned to pre-tilt levels, after 10 min. Time to recovery was recorded as outcome data.

Startle Response (8 min): The startle stimulus consisted of a 50 msec burst of 105 dB white noise. On half the 20 trials, the startle stimulus was preceded by a 50 msec tone pip at 440 Hz and 90 dB; the rise/fall time was 25 msec. The volunteer was instructed to listen to the tones, and count the double tones. Electromyographic (EMG) measures from the orbicularis oculi provided quantitative information for analysis of the amplitude of the startle reflex and its inhibition (PPI).

Signal processing.

The digital ECG data was analyzed using custom software previously described[56]. Analyses followed consensus guidelines[57] and were performed for spectral total power (0.003 Hz to 0.4 Hz), absolute power in the low (ABS LF, 0.04 Hz to < 0.15 Hz) and high (ABS HF, 0.15 Hz to 0.4 Hz.) frequency bands, percent total power in the LF and HF bands (%LF, %HF, respectively), and the LF/HF ratio. Mean BP was derived from the arithmetic average of the digitized BP signal. SBP and DBP were quantified from the continuous digitized record of the tonometric BP signal.

Statistical analysis

Analysis of Variance (ANOVA; BMDP4V) for mixed designs was performed separately for each task, and for specific outcome measures within tasks. All results for DC and NDC Control groups were compared. If no differences were found, the groups were combined for comparison to Cases; Group (Cases, Controls) was the between-subjects factor, and Period (baseline, task) was the within-subjects factor. When the Control groups differed, all three groups were used as the between-subjects factor. Because differences in sample size were not random, the "size" option for calculating the ANOVA, which adjusts F values for such differences, was employed. Probability values were corrected for lack of sphericity using the Huynh-Feldt epsilon technique, and statistical significance was set at $p < 0.05$. Three veterans (one from each group) did not participate in the tilt test because of the *a priori* PVC elimination criteria of one or more PVC per minute during the initial baseline, no participants were excluded from tilt testing because of the BP criteria. Thirteen (7 Cases, 1 DC, 5 NDC) showed signs of syncope and terminated early, and data for another was invalidated by equipment malfunction. To use as much data as possible, the first 5-min of head-up tilt was analyzed for all 89 veterans who completed the initial portion of the task; the 75 veterans with complete data were included in an analysis of all 5-min head-up tilt periods.

2.2 Results. Study 1: Epidemiologic, Exposure and Genetic Results

2.2.1 Volunteers

Volunteers in this study were recruited from Gulf War veterans who lived in the Kansas City area. The majority of the volunteers were part of the KVP database. Additional volunteers were identified using a database of veterans who resided in Missouri. The KVP database has been described previously[17]. Potential volunteers were screened over the phone to determine whether or not they met criteria for inclusion into the study. Volunteers who passed this screening were classified as Case or Control based on their responses to questions concerning symptoms; the Centers for Disease Control and Prevention (CDC) Case definition criteria was used for the classification. Recruitment continued until approximately equal numbers of Cases and Controls were enrolled into the study and the total enrollment number reached at least 300. Questionnaire data and blood samples were collected from 304 individuals; of whom 144 (47%) were identified as Cases and 160 (53%) were identified as Controls at the time of screening interview. Our original targets had been 150 Cases and 150 Controls, so the recruitment effort was successful.

The sample reported here was primarily: male (93%), white (89%), enlisted personnel (79%), Army personnel (55%), active duty military (66%) rather than Reserve or National Guard, and enlisted at the time of the Gulf War (79%). At the time the study data were collected (Fall, 2000), the average age of the sample was 38 yrs (range 28 to 64), 24% were still in the military, and 88% had education above a high school level.

Measures

Analyses presented in this report consist primarily of the relation between the genotype classification, self-report symptom data, and classification as Case or Control using both the CDC criteria and the KVP criteria[24]. Each volunteer provided a blood sample for genetic analysis and completed a questionnaire to provide information on Gulf War experience exposures while deployed in the Gulf, symptoms that have been experienced, medical conditions, and demographics.

Self-report

Each volunteer completed a questionnaire concerning: military service between August, 1990 and July, 1991, including time in the Gulf area, location of deployment, exposures while in the Gulf, and military assignment and occupation; symptoms experienced during the past six months including severity (mild, moderate, severe) and timing of first occurrence of the symptom (i.e., before Gulf deployment vs. after Gulf deployment); general health status; and demographics. The symptom list included items required to determine Case/Control status for both the CDC and KVP classification system, as well as items that reflect various dysfunctions of the ANS. An 8-item scale, that reflects ANS dysfunction, was developed by summing the severity response (no experience, mild, moderate, severe) to the symptoms that reflect ANS dysfunction. These items included: breathing stops for a few seconds while sleeping; loud snoring; dizziness or faintness; sweating an unusual amount; night sweats; heart racing or pounding; feeling dizzy or light-headed when standing up; and gastrointestinal (GI) symptoms such as diarrhea, nausea, or abdominal pain. The ANS scale had acceptable reliability

(Cronbach's alpha = .750), and it was not necessary to perform a transformation to improve the scale's distributional properties.

Case/Control Classification

Based on symptom self-report data provided in the questionnaire, each volunteer was classified as a Case or Control using both the CDC criteria for GWI[24] and also the KVP criteria for GWI[32]. Standard scoring procedures were followed to arrive at the GWI Case/Control classification using the CDC criteria. Volunteers were classified as a Case if they reported symptoms in at least two of the following three categories of symptoms: fatigue; pain (muscle pain, joint pain, joint stiffness); and mood/cognitive (problems getting to sleep or staying asleep; difficulty concentrating, difficulty remembering recent information, trouble finding words when speaking, feeling moody, feeling anxious, feeling down or depressed).

The KVP criteria for Case/Control classification takes into account the timing of the reported symptoms. In order to be classified as a GWI Case, symptoms that are reported must have begun either during or after Gulf deployment rather than before deployment. Case classification was based on reporting either moderate or severe symptoms, or multiple mild symptoms, in at least 3 of the following symptom groupings: fatigue (fatigue, feeling unwell after physical exercise or exertion, problems getting to sleep or staying asleep, not feeling rested after sleeping); pain (joint pain, muscle pain, body pain where you hurt all over); neurological (headaches; feeling dizzy; lightheaded, or faint; eyes very sensitive to light; blurred or double vision; numbness or tingling in your extremities; tremors or shaking; low tolerance for heat or cold; night sweats; having physical or mental symptoms after breathing in certain smells or chemicals; difficulty concentrating; difficulty remembering recent information; trouble finding words when speaking; feeling down or depressed; feeling irritable or having angry outbursts); skin (skin rashes, other skin problems); gastrointestinal (diarrhea, nausea or upset stomach, abdominal pain or cramping); and respiratory (difficulty breathing or catching your breath, frequent coughing when you don't have a cold, wheezing in your chest).

Statistical Methods

The questionnaire data were examined for completeness and validity of responses. Distributional properties of all continuous measures were examined to determine whether transformations were necessary to meet statistical assumptions. Statistical analyses were completed after receipt of genetic data from the analysis of the blood samples. The primary statistical analysis techniques used were chi square analysis, and comparison of means using either analysis of variance (ANOVA) or independent groups t-test, using BMDP software. For each comparison of means using independent groups t-test, the equality of the variances of the groups was examined and the appropriate statistic is reported.

Comparison of Case/Control Classification Using CDC and KVP Definitions

Volunteers were recruited into the study and assigned an initial Case/Control classification, using the CDC definition, based on their responses to the screening interview. Case/Control classification for purposes of analyses were made using responses to the questionnaire.

As expected, there was significant agreement on Case/Control classification between the KVP and CDC definitions (83% agreement; kappa = .656, p < .0001, 95% confidence limits .574 - .736). There were discrepant classifications for only 53 of the 304 volunteers. As has been found previously[24], the majority of these discrepancies (48 of 53) were the result of Case classification using the CDC criteria while the KVP definition resulted in a Control classification.

Given our hypotheses about potential ANS involvement in GWI, we examined in detail the subset of the questionnaires that dealt with ANS symptoms. Scores on the ANS scales were compared for Cases and Controls using both Case definitions. Cases had a significantly higher report of ANS-related symptoms compared to the Controls (KVP definition: T = 12.37, df 302, p < .0001, 6.9 vs. 2.0; CDC definition: T = 11.68, df 302, p < .0001, 6.0 vs. 1.7).

2.2.2 Relationship Between Butyrylcholinesterase Genetics and Case/Control Classification

Table 1 presents the distribution of the genetic status of BChE by Case/Control classification. The top half of the table presents the Case/Control classification using the CDC definition, and the bottom half presents the data distribution using the KVP definition. The upper row gives the genetic assignment. It must be remembered that some mutations in BChE will often appear together in one allele; for example, the A and K mutations will often appear together in one allele, accounting for the ten U/AK and the one AK/F volunteers. U refers to the "usual" or wild-type form of the enzyme.

Table 1. Distribution of Case versus Control for each genetic category

Genetics	U/U	U/K	K/K	U/AK	U/A	A/F	AK/F	Total
CDC								
Case	115	54	8	6	2	1	1	187
Control	74	33	5	4	1	0	0	117
KVP								
Case	89	41	7	5	1	0	1	144
Control	100	46	6	5	2	1	0	160
Total	189	87	13	10	3	1	1	304

The simple form of the hypothesis, from Dr. Lockridge's earlier work[18], that heterozygote carriers of the A and F mutations and homozygote carriers of the K mutation of BChE are more likely to report symptoms and be Cases was not supported with regard to Case/Control classification. The overall proportion of Cases was 62% using the CDC definition and 47% using the KVP definition. This general distribution was typically maintained for each of the genetic categories that had a large enough sample size to evaluate. As noted below, however, more detailed analyses uncovered strong, significant associations between the K/K genotype and specific symptom scores.

Relationship Between BChE Genetics and Symptom Report

The genetic hypothesis was also tested by examining the relationship between genetic status of BChE and reported symptoms. While there were no significant differences in symptom report when all of the seven genotypes present in our population were compared, there were differences when the genotypes were grouped. Volunteers were grouped into one or the following three genetic classifications, based on the degree of enzyme hydrolytic velocity: (1) U/U or U/K, (2) K/K, and (3) U/AK, U/A, A/F, or AK/F (to be referred to as the [AKF] group).

Volunteers in the K/K group reported significantly more symptoms related to gastrointestinal symptoms (i.e., diarrhea, nausea/upset stomach, abdominal pain or cramping) than did volunteers in either the [UU-UK] or the [AKF] group ($F = 4.00$, df 2, 301, $p < .02$; 2.46 vs. 1.05 and .53). The KK group also reported more respiratory symptoms (i.e., difficulty breathing or catching breath, frequent coughing without a cold, wheezing in chest) than did either the [UU-UK] or the [AKF] group ($F = 3.29$, df 2, 301, $p < .04$; 1.85 vs. .74 and .93); the difference between the K/K and the [UU-UK] groups was significant ($p < .03$). There was also a trend for the K/K group to report more fatigue symptoms (i.e., fatigue, feeling unwell after exercise or exertion, problems getting to sleep or staying asleep, not feeling rested after sleep) than the other two groups ($F = 2.48$, df 2, 301, $p < .09$; 4.85 vs. 2.97 and 2.33). The difference between the K/K group and the other groups with respect to GI and respiratory symptoms is significant. It is not present if a volunteer is carrying only one copy of the K allele, whether in the comparisons with the [AKF] pooled group or the UK group. Subgroup analyses of the U/K volunteers showed they were statistically indistinguishable from the U/U group.

2.2.3 Relationship Between Case/Control Classification and Exposure

As would be expected from the fact that Case/Control classification is based on the reporting of symptoms, these two groups differed significantly from each other on all symptom items. In order to understand the possible role that exposure might play in symptom report, additional analyses were conducted to examine the relationship between Case/Control classification and the exposures to potentially stressful factors. Cases were significantly more likely than Controls to report exposure to a wide variety of agents. Table 2 shows the proportion of each group that reported experiencing each kind of exposure. Only those items with significant differences using both the KVP and the CDC Case/Control definitions are included in the table; the percentages and p values reported are based on the KVP definition of Case/Control.

Table 2. Comparison of Exposures Between Cases and Controls

Exposure	% Cases	% Controls	P ≤
Saw Iraqis or civilians who had been badly wounded or killed	65	40	.0001
Handled or came into contact with POWs	59	35	.0001
Came into direct contact with destroyed enemy vehicles	60	36	.0001
Used pesticide cream or spray on skin	57	31	.0001

Exposure	% Cases	% Controls	P ≤
Took PB pills	72	44	.0001
Frequently had less than 4 hrs of sleep in a 24-hr period	69	49	.001
Smoke from oil well fires	82	65	.001
Saw or came into contact with dead animals	54	34	.001
Had SCUD missile explode within one mile	48	31	.002
Saw destroyed enemy vehicles	74	58	.003
Received one or more shots in the arm while in theater	73	58	.006
Received one or more shots in the buttocks while in theater	43	29	.02

Additional analyses were conducted to explore the relationship between sleep loss and Case/Control status separately for specific genetic groups. The relationship was significant for those volunteers who were in the [UU-UK] genetic group ($p < .01$ and $p < .02$ for the KVP and CDC definitions, respectively). The relationship was stronger for those in the K/K group ($p < .0005$ and $p < .003$ for the KVP and CDC definitions, respectively). Regardless of which Case/Control definition is used, every volunteer with the K/K genotype who reported frequently having less than 4 hrs of sleep in a 24-hr period is a Case. This clear split in the distribution was not found for either the [UU-UK] or the [AKF] genetic groups.

Other analyses explored whether these specific associations with the K/K genotype could be explained by the known differences in enzymatic hydrolytic velocity between the wild-type enzyme and the various identified mutations. As expected, there were significant differences in activity level among the seven genotypes in our sample ($F = 15.71$, df 6, 297, $p < .0001$).

Genotype	Mean Enzyme Activity (μmoles benzoylcholine per min per mL)
U/U	1.19
U/K	1.01
K/K	0.78
U/AK	0.76
U/A	1.03
A/F	0.92
AK/F	0.69

Thus, in terms of enzyme velocity U/U > U/K, K/K, U/AK, but genotype U/K > K/K, U/AK. K/K does not differ from any of the other mutant groups (i.e., U/AK, U/A, A/F, A/KF). The finding with respect to symptoms held up when the genotypes were grouped as: U/U vs. U/K vs. K/K vs. (U/AK, U/A, A/F, AK/F) ($F = 30.03$, df 3, 303, $p < .0001$). Thus, the differences in symptoms reported by the K/K volunteers did not correlate with mean enzyme activity.

The use of a questionnaire that permitted clear Case-Control assignment and a large sample size allowed us to uncover several other significant associations. Insights were obtained using a classification based on enzyme velocity to obtain a dichotomous classification. We combined the

genotypes U/U and U/K as our "nonvariant group" and the remaining genotypes (K/K, U/AK, U/A, A/F, or AK/F) as the "variant group." As shown in Table 3, the results indicated that illness risk associated with certain exposures was particularly pronounced among variant volunteers. For example, the odds ratio associated with PB exposure was 40.0 (95%CI = 3.58 - 447.04) for the variant group, while it was much lower, 2.68 (95%CI = 1.62 - 4.44) for the nonvariant group. For this exposure, the difference between PB-associated risk in variants vs. nonvariants is significant at the 0.02 level by the Breslow-Day test for homogeneity of odds ratios.

Table 3. Genetic Variant Status and Exposures in Association with Case Status

Exposures in Theater	All Volunteers (n = 304)			Genetic Variant Volunteers Only (n=28)			Nonvariant Volunteers (n= 276)			Tests for Variants vs. Nonvariants	
	%Cases	%Controls	OR(95%CI)	%Cases	%Controls	OR(95%CI)	%Cases	%Controls	OR(95%CI)	BD[1] p value	Interaction[2] p value
Participated in ground combat			1.42			8.00			1.18		
Yes	32%	25%	(0.86-2.36)	57%	14%	(1.28-	30%	26%	(0.69-2.02)	0.041	0.050
No	68%	75%		43%	86%	50.04)	70%	74%			
Witnessed Iraqi or civilian casualties			2.71			15.00 (2.26-			2.34 (1.44-3.80)	0.054	0.063
Yes	65%	40%	(1.70-4.31)	86%	29%	99.64)	62%	41%			
No	35%	60%		14%	71%		38%	54%			
Contact with POWs			2.62			15.00			2.26		
Yes	59%	35%	(1.64-4.17)	86%	29%	(2.26-	56%	36%	(1.39-3.67)	0.049	0.058
No	41%	65%		14%	71%	99.64)	44%	64%			
Saw or had contact with dead animals			2.20			36.00			1.75	0.003	0.006
Yes	53%	34%	(1.38-3.51)	86%	14%	(4.33-	50%	36%	(1.07-2.85)		
No	46%	66%		14%	86%	299.02)	50%	64%			
Received shot(s) in arm while in theater			2.00			*			1.71	0.028	*
Yes	73%	58%	(3.21-1.97)	100%	54%		70%	58%	(1.02-2.86)		
No	27%	42%		0	46%		30%	42%			

Exposures in Theater	All Volunteers (n = 304)			Genetic Variant Volunteers Only (n=28)			Nonvariant Volunteers (n= 276)			Tests for Variants vs. Nonvariants	
	%Cases	%Controls	OR(95%CI)	%Cases	%Controls	OR(95%CI)	%Cases	%Controls	OR(95%CI)	BD[1] p value	Interaction[2] p value
Took PB Yes No	72% 28%	44% 56%	3.21 (1.97-5.24)	92% 8%	23% 77%	40.00 (3.58-447.04)	69% 31%	46% 54%	2.68 (1.62-4.44)	0.019	0.032
Frequently had < 4 hrs sleep in 24 hrs Yes No	69% 31%	49% 51%	2.04 (1.14-3.63)	83% 16%	21% 79%	18.33 (2.52-133.26)	67% 33%	52% 48%	1.89 (1.15-3.08)	0.022	0.029

[1] Breslow-Day test for homogeneity of odds ratios: association of exposures with Case status, stratified by variant status
[2] Maximum likelihood logistic regression model testing interaction between variant status and exposure in predicting Case status
* Undefined; 0 cell value

In summary, the original form of the hypothesis[25], that heterozygote carriers of the A and F mutations and homozygote carriers of the K/K mutation of BChE would be present in a higher frequency in the Cases than in the Controls, was not supported. However, the use of a questionnaire that permitted clear Case-Control assignment (by either the CDC or the KVP criteria) and a large sample size allowed us to uncover a significant association between the K/K genotype and Case status and GI and respiratory symptom scores, as well as a significant association between that same genotype and reported sleep loss. These results do not correlate with the enzyme velocity, and are not present in the volunteers who only have one copy of the K allele, regardless of whether the other copy has a normal velocity (U) or has one or more other mutations (A, F, or AK).

2.2.4 Carbamate Affinity Testing

We examined whether Cases and Controls differed in their affinity (as reflected in the K_{app} of pyridostigmine for AChE) for the carbamate PB. If they did, this could explain differential sensitivity of Cases and Controls to the exposure of "taking NAPP pills," which is the field nomenclature for PB. The results indicated no differences between Cases (K_{app} = 0.162 uM (SEM = 3.95 nM)) and Controls (K_{app} = 0.161 uM (SEM = 3.45 nM)).

2.2.5 Other Genetic Findings

In addition to the above results, we discovered a new naturally-occurring mutation, Asp70His, in human BChE. As noted above, we phenotyped 304 Gulf War veterans, since some genetic assignments can be made unambiguously with appropriate phenotyping. We also examined 4 nonveteran internal Controls. In addition, we genotyped all of the suspected K mutations. Serum samples were phenotyped by measuring activity with benzoylcholine, and inhibition of activity by dibucaine, sodium fluoride, and the Roche compound RO 2-0683. In the first annual report we noted that one sample had "not worked out in two attempts." One sample, from a veteran, out of the 308 was found whose inhibition values did not match the values for any of the known genetic variants of human BChE. The serum had an activity of 0.96 μmoles benzoylcholine hydrolyzed per minute per ml, similar to the activity of 1.2 μmoles per min per ml for 191 wild-type samples in the group. However, its dibucaine number of 42, fluoride number of 24, and Roche number of 33 were a novel set. Our initial interpretation was that the genotype was A/F with one allele containing the D70G (atypical - A) mutation and the other a new, hitherto unreported Fluoride variant. However, DNA sequencing showed that this interpretation was incorrect.

A single mutation was found in one allele. Codon 70 had C in place of G, thus changing Asp 70 (GAT) to His (CAT), nucleotide 208G->C. No other mutations were found in the coding region. The presence of the mutation was confirmed by repeating the PCR and sequencing in both directions. To obtain the D70H mutant in a homozygous state, the D70H mutant was transiently expressed in 293T human embryonic kidney cells and the secreted BChE collected into serum-free medium. The dibucaine number of the homozygous D70H was 31, the fluoride number was 13, and the Roche number was zero.

The catalytic constant (kcat) value for benzoylcholine was determined by measuring maximal velocity (Vmax) and titrating the active sites with chlorpyrifos oxon. The kcat value for D70H was found to be higher than that for wild-type BChE, 18-25,000 min-1 rather than 15,000 min-1. The Km value for benzoylcholine was 46 μM for D70H, 5 μM for wild-type, and 27 μM for D70G.

The D70G atypical allele is carried by 1 out of 25 Caucasians. The D70H allele is expected to have a 50 fold lower frequency, because D70H has been found only once in 50 atypical alleles sequenced from unrelated individuals. Fifteen atypical alleles are from the present work and 35 from previous work. This newly-discovered D70H mutation brings to 40 the total number of naturally occurring BChE mutations identified in the human population.

People homozygous for the atypical (A) variant, D70G, always respond with prolonged apnea to a normal dose of succinylcholine or mivacurium. Since the D70H variant has an even poorer binding affinity than D70G, it is expected that people homozygous for D70H will also experience prolonged apnea. These results on D70H appeared the Annals of Clinical Biochemistry[58].

2.3 Results. Study 2: Epidemiologic Studies and Exposure Assessment

2.3.1 Study Population

A very high proportion of veterans who were contacted for the study agreed to participate, and all recruitment targets were met or exceeded. Contact attempts were made with 353 veterans for the Study 2 Case/Control sample. Of those, no working telephone number was identified for 81 veterans (23%), 19 (5%) were deployed or had moved out of the area, 3 veterans were deceased and 1 veteran was too disabled to be interviewed. Telephone contact was made with the remaining 249 veterans, of whom 11 (4%) refused to be interviewed and 43 (17%) were ineligible for the interview. Of the 43 ineligible veterans contacted, 27 were not in one of the target units, 6 were sampled as nondeployed, but reported serving in the Gulf War, 4 were sampled as Gulf War veterans, but reported not serving in the war, and 5 were sampled as nondeployed era veterans, but said they had not served in the military during the index period.

Overall, of the 206 veterans who were contacted and found to be eligible for the screening interview, 195 (95%) completed interviews. Of the 195 screened veterans, 30 were found to be ineligible for the main study due to medical exclusions, 37 were ineligible because they were Controls contacted after recruiting for Controls had closed, and 3 were ineligible because they were nondeployed veterans, but met Case criteria. Of the 125 determined to be eligible for the study and invited to schedule an appointment at the testing site, 113 (90%) agreed and 12 (10%) declined. Of those who agreed to participate, 93 (82%) completed their appointment.

The final Study 2 Case/Control sample consisted of 42 volunteers provisionally characterized as Gulf War-deployed Cases using the KVP Case definition applied to telephone screening data, 26 volunteers provisionally characterized as DC, and 25 NDC. As reported for Study 1, some veterans endorsed more symptoms when filling out the questionnaire than they had in the screening interview, resulting in some differences between Case/Control status assigned using telephone interview data vs. questionnaire data. To maintain consistency between symptom data and other data collected for the study, questionnaire symptom data were used to assign final Case/Control status. This resulted in a reassignment of Case/Control status for 11 volunteers, including 2 volunteers who had been originally recruited as nondeployed Controls. Because nondeployed Cases were not eligible for the study, these 2 volunteers were dropped from the analytic sample.

Thirty-one volunteers who participated in Study 1 also participated in Study 2. This included 23 of 28 BChE variants (homozygous for the K mutation, or heterozygous carriers of the A or F mutations) identified in Study 1, referred to here as the variant sample. Based on the enzyme velocity, homozygous carriers of the wild-type U allele (U/U), or U/K heterozygotes were combined, as described in Study 1, into the nonvariant sample. Study 2 also included 8 volunteers who had participated in Study 1 who were not carriers of BChE mutations, but were Army enlisted personnel who had served in one of the two target units, and so were eligible to participate as Case/Control volunteers in Study 2 and were recruited and screened as described for the entire Case/Control sample.

Actual Sample: The final analytic samples for Study 2 included 91 volunteers in the Case/Control sample: 49 Cases (which exceeded the planned 40), 19 DC (six less than the planned 25) and 23 NDC (two less than the planned 25). The final variant sample recruited from Study 1 were all Gulf War-deployed veterans and included 11 variant Case volunteers and 12 variant Control volunteers (three over the planned 20). The two categories where we were short from the planned goal resulted from reassignments of Case/Control status due to differences in symptom reporting between the written questionnaire and the screening interview.

2.3.2 Characteristics of Study 2 Case/Control and Variant Samples

Table 4 compares military and demographic characteristics of Case and Control volunteers in the Study 2 Case/Control sample. As designed, the sample consisted entirely of veterans who had served as Army enlisted personnel during the war, with approximately 10% of each group being female. Cases differed from DC only in the proportion who had come from reserve units, with a higher proportion of DC being reservists. The majority of veterans (76-80%) were no longer in the military. NDC were also very similar to DC, in terms of military and demographic characteristics. Only total annual income was significantly different between the two groups ($p = 0.04$), with a higher proportion of NDC reporting a household income under $35,000.

As shown in Table 5, Cases and Controls reported similar general health status prior to the war, but a significantly higher proportion of Cases (55% vs. 0% Controls; $p < 0.01$) reported their health as fair or poor at the time of the study. All Control volunteers reported their current health status as good to excellent. Cases also scored significantly higher on the ANS scale generated for Study 1 ($p < 0.01$), consisting of summed severity scores for self-reported symptoms adapted from the Mayo Autonomic Symptom Profile. Interestingly, Cases were significantly more likely to report themselves as regular smokers during the war ($p < 0.01$), but not at the time of the study. Cases were also similar to Controls with respect to BChE genotype distribution.

The SF-36 includes two subscales, one for the physical components of self-reported symptoms, and one for the mental components. Lower scores indicate more symptoms. Cases had lower scores on the physical component than either Control group ($F = 29.65$, df 2, 87, $p < .0001$; Cases = 43, DC = 54, NDC = 56). Similar results were found for the mental component ($F=10.55$, df 2, 87, $p < .0001$; Cases = 47, DC = 56, NDC = 55).

As expected, there was more heterogeneity between variant Cases and variant Controls, since these volunteers were recruited based on their genotype, and not on military, demographic, or health status parameters (Table 6). Also of note, as with the Case/Control recruited sample, variant Cases were significantly more likely to report themselves as smokers during the war, but not at the time of the study (Table 7). Overall, the variant sample was also distinct from the Case/Control sample in terms of veterans' military and demographic characteristics, with differences in rank, branch of service, military component, age, marital status, race, and education (Table 8).

Variant volunteers had lower SF-36 physical component scores than variant Controls ($F = 63.45$, df 1, 109, $p < .0001$; variants = 44, nonvariants = 55). Similar results were found for the mental component scores ($F = 27.24$, df 1, 109, $p < .0001$; variants = 44, nonvariants = 54).

Table 4. Military and Demographic Characteristics of Study 2 Case/Control Sample

	Cases vs. All Controls			DC vs. NDC		
	% Cases (n=49)	% Controls (n=42)	p* value	% DC (n=19)	% NDC (n=23)	p* value
Rank: Enlisted	100	100	-	100	100	-
Branch: Army	100	100	-	100	100	-
Component:						
Regular	98	71		83	61	
Reserves	2	24	<0.01	17	30	0.22
Guard	0	5		0	9	
Still in military?						
No	80	76	0.70	68	83	0.28
Yes	20	24		32	17	
Sex: Male	90	90		89	91	
Female	10	10	0.91	11	9	0.84
Age: 29-34	10	21		26	17	
35-39	31	36		32	39	
40-44	20	14	0.52	21	9	0.45
45-49	18	14		16	13	
50+	20	14		5	22	
Marital status						
Married	88	74		74	74	
Divorced	10	12	0.08	10	13	0.95
Single	2	15		16	13	
Employment status						
Employed full time	92	95		95	96	
Employed part time	0	5	0.22	5	4	0.89
Seeking work	4	0		0	0	
Student	2	0		0	0	
Retired	2	0		0	0	
Hispanic ethnicity						
No	90	93	0.61	100	87	0.10
Yes	10	7		0	13	
Race White	61	76		89	65	
Black	29	19	0.29	11	26	0.15
Other	10	5		0	9	
Education						
High school	12	7		5	9	
Some college	71	62	0.23	74	52	0.40
4 year degree	16	26		21	30	
> 4 year degree	0	5		0	9	
Income $20-34,999	31	14		0	26	
$35-50,000	32	45	0.16	58	35	0.04
> $50,000	37	40		42	39	

*p values indicate probabilities using chi squared tests, except when a 0 value was contained in a cell, where Fisher's exact test was used.

Table 5. Health-Related Characteristics of Study 2 Case/Control Sample

	Cases vs. Controls			DC vs. NDC		
	% Cases (n=49)	% Controls (n=42)	p* value	% DC (n=19)	% NDC (n=23)	p* value
Health status in 1990						
Excellent	53	53		53	39	
Very Good	41	47	0.52	47	48	0.23
Good	6	0		0	13	
Health status at time of study						
Excellent	0	10		10	18	
Very Good	4	58		58	35	
Good	41	32	<0.01	32	48	0.32
Fair	47	0		0	0	
Poor	8	0		0	0	
Regular smoker before deploymt/1990	61	37	0.07	37	35	0.89
Regular smoker during deployment	63	21	<0.01	21	-	
Regular smoker at time of study	35	26	0.51	26	35	0.55
Genotype						
UU	61	63		63	74	
UK	33	32	0.93	32	26	0.47
KK	4	5		5	0	
UAK	2	0		0	0	
Mean ANS scale score	6.69	0.47	<0.01	0.47	0.61	0.62

*p values indicate probabilities using chi squared tests, except when 0 value was contained in cell, where Fisher's exact test was used.

Table 6. Military and Demographic Characteristics of Variant Sample

	Variant Cases vs. Variant Controls		
	% Variant Cases (n=11)	% Variant Controls (n=12)	p* value
Rank: Enlisted	100	75	
Officer	0	25	0.08
Branch: Army	64	50	
Navy	9	17	
Air Force	0	8	0.71
Marines	27	25	
Component:			
Regular	64	58	
Reserves	36	33	0.62
Guard	0	8	
Still in military?			
No	73	83	
Yes	27	17	0.54
Sex: Male	100	83	
Female	0	17	0.16
Age: 29-34	45	42	
35-39	27	8	
40-44	9	8	0.41
45-49	18	17	
50+	0	25	
Marital status			
Married	64	75	
Divorced	9	25	0.12
Single	27	0	
Employment status Employed full time	100	100	-
Hispanic ethnicity			
No	100	100	-
Yes	0	0	
Race White	91	100	
Black	0	0	
Native American	9	0	0.29
Other	0	0	
Education			
High school	27	25	
Some college	27	33	0.92
4 year degree	27	17	
> 4 year degree	18	25	
Income $20-34,999	27	17	
$35-50,000	27	25	0.78
> $50,000	45	58	

* p values indicate probabilities using chi squared tests, except when 0 value was contained in cell, where Fisher's exact test was used.

Table 7. Health-Related Characteristics of Variant Sample

	Variant Cases vs. Variant Controls		
	% Variant Cases (n=11)	% Variant Controls (n=12)	p* value
Health status in 1990			
Excellent	55	83	
Very Good	45	17	0.13
Good	0	0	
Health status at time of study			
Excellent	0	8	
Very Good	9	42	
Good	9	50	<0.01
Fair	55	0	
Poor	27	0	
Regular smoker before deployment/1990	36	8	0.10
Regular smoker during deployment	55	8	0.02
Regular smoker at time of study	18	17	0.92
Genotype			
U/U	0	0	
U/K	0	0	
K/K	55	25	
U/AK	27	58	0.16
U/A	18	0	
A/F	0	8	
AK/F	0	8	
Mean ANS scale score	7.27	2.00	<0.01

* p values indicate probabilities using chi squared tests, except when 0 value was contained in cell, where Fisher's exact test was used

Table 8. Military and Demographic Characteristics of Study 2 Case/Control Sample vs. Variant Sample

	Study 2 Cases vs. Variant Cases			Study 2 Controls vs. Variant Controls		
	% Study 2 Cases (n=49)	% Variant Cases (n=11)	p* value	% Study 2 Controls (n =42)	% Variant Controls (n=12)	p* value
Rank: Enlisted	100	100		100	75	
Officer	0	0	-	0	25	<0.01
Branch: Army	100	64		100	50	
Navy	0	9	<0.01	0	17	<0.01
Air Force	0	0		0	8	
Marines	0	27		0	25	
Component:						
Regular	98	64		71	58	
Reserves	2	36	<0.01	24	33	0.71
Guard	0	0		5	8	
Still in military?						
No	80	73	0.62	76	83	0.60
Yes	20	27		24	17	
Sex: Male	90	100		90	83	
Female	10	0	0.57	10	17	0.49
Age: 29-34	10	45		21	42	
35-39	31	27		36	8	
40-44	20	9	0.05	14	8	0.31
45-49	18	18		14	17	
50+	20	0		14	25	
Marital status						
Married	88	64		74	75	
Divorced	10	9	0.02	12	25	0.25
Single	2	27		14	0	
Employment status						
Employed full time	92	100		95	100	
Employed part time	0	0	0.81	5	0	0.44
Seeking work	4	0		0	0	
Student	2	0		0	0	
Retired	2	0		0	0	
Hispanic ethnicity						
No	90	100	0.27	93	100	0.34
Yes	10	0		7	0	
Race White	61	91		76	100	
Black	29	0		19	0	
Native American	0	9	0.02	0	0	0.17
Other	10	0		5	0	
Education						
High school	12	27		7	25	
Some college	71	27	0.01	62	33	0.04
4 year degree	16	27		26	17	
> 4 year degree	0	18		5	25	
Income $20-34,999	31	27		14	17	
$35-50,000	32	27	0.86	45	25	0.44
> $50,000	37	45		40	58	

*p values indicate probabilities using chi squared tests, except when 0 value in was contained cell, where Fisher's exact test was used.

2.3.3 Association of Case Status With Deployment Locations and Exposures

Veterans filled out a study questionnaire that included a map of the Persian Gulf Theater of Operations with several geographical areas delineated, and reported whether they had been in each area, and for how long. In contrast to our findings in Study 1 and in a large, population-based study of Kansas veterans[24], there were, overall, few associations between geographical location and Case status. This may be due to the fact that nearly all veterans in the study reported spending time in Iraq, Kuwait, and Eastern Saudi Arabia, areas shown in the earlier study to be linked to the highest illness rates. In the present study, Cases were significantly more likely to report spending one week or longer in the island nation of Bahrain, but not any other locations (Table 9).

Also in contrast to many previous studies, including our Study 1 results, very few associations were found between self-reported exposures in theater and Case status (Table 10). Cases were not more likely to report any in-theater exposures associated with stress or trauma, such as hearing chemical alarms or witnessing deaths or serious injuries among U.S. troops, or Iraqi troops or civilians. In fact, Cases were significantly *less* likely than Controls to report witnessing U.S. casualties in theater for one week or longer.

In the Case/Control sample, bivariate analyses identified only 3 significant risk factors for Gulf War illnesses: using pesticide cream on the skin for one month or longer, wearing uniforms treated with pesticides for one week or longer, and taking PB tablets for one week or longer. This is notable for two reasons. First, all three of these findings may reflect exposures that may alter cholinergic transmission and are therefore particularly relevant to our study hypotheses. Second, the dearth of identified associations between illness and exposures suggests that ill veterans in this study did not systematically over-report in-theater exposure experiences, making the identified links between illness and exposures more credible.

There were also few significant differences in exposure histories reported by Cases and Controls in the variant sample, although small numbers in this sample made it unlikely that any but the very largest differences would be detected. Still, there was a strong and highly significant association between taking PB ever during deployment and being a variant Case, with 91% of variant Cases reporting having used PB pills, and only 27% of variant Controls. In addition, variant Cases were significantly more likely to report seeing or having contact with dead animals for at least 7 days while in theater (Table 10).

As summarized in Table 11, additional analyses were conducted to explore the possibility that combinations of exposures would have a greater association with illness than individual exposures. Therefore, grouped variables were constructed which combined variables possibly associated with exposure to chemical agents (heard chemical alarms, and saw dead animals), possible exposure to depleted uranium (contact with destroyed enemy vehicles or with U.S. vehicles destroyed by friendly fire), experiences that might have been extremely stressful or traumatic (heard chemical alarms, had a SCUD missile explode within 1 mile, engaged in ground combat, saw U.S. or Iraqi deaths or serious injuries), and pesticide use (on skin, on uniforms, wore flea collar, living area sprayed). In the Case/Control sample, none of these grouped variables were significantly associated with illness. However, among genetic variants, significant associations were found between illness and having one or more traumatic experiences, and being exposed to PB plus possible exposure to chemical agents. Also

in this group, a strong, but nonsignificant risk was seen for the combination of PB and pesticide exposure. Given the small numbers in the variant sample, these findings are intriguing, although inconclusive.

Veterans were also asked about immunizations (shots) they had received from the military during the time of the Gulf War. Of the 8 shots queried, only receipt of typhoid and yellow fever vaccines were significantly associated with illness (Table 12). Sixteen of the 50 veterans in the deployed Case/Control sample reported using their shot records to answer this group of questions. Despite these small numbers, receipt of typhoid, yellow fever, and plague vaccine were found to be significantly associated with Case status. However, reported receipt of these shots were highly intercorrelated which, in addition to the very small sample, makes a clear interpretation of risk associated with any single vaccine impossible.

In the variant sample, only self-reported receipt of botulinum toxoid vaccine was significantly associated with illness. (Table 13) Again, however, small sample size made the presence or absence of significant associations difficult to interpret.

Table 9. Association of Deployment Locations With Case Status

	Study 2 Cases vs. Controls			Study 1 Variant Cases vs. Study 1 Variant Controls		
	% Cases (n=49)	% Controls (n=19)	p* value	% Variant Cases (n=11)	% Variant Controls (n=12)	p* value
Eastern Saudi Arabia						
Ever	98	89	0.13	91	67	0.16
1 wk or longer	92	89	0.76	82	50	0.11
1 mo or longer	53	42	0.42	64	50	0.51
Bahrain						
Ever	70	61	0.48	45	50	0.83
1 wk or longer	60	28	0.02	18	8	0.48
1 mo or longer	13	11	0.86	9	0	0.48
Kuwait						
Ever	94	89	0.53	55	50	0.83
1 wk or longer	73	68	0.68	36	33	0.88
1 mo or longer	18	11	0.43	18	0	0.22
Iraq						
Ever	80	84	0.66	55	42	0.54
1 wk or longer	69	68	0.94	45	33	0.55
1 mo or longer	20	21	0.95	9	8	0.95
Northern Saudi Arabia						
Ever	41	44	0.82	36	25	0.55
1 wk or longer	28	33	0.69	36	17	0.28
1 mo or longer	7	22	0.07	9	0	0.48
Central Saudi Arabia						
Ever	68	53	0.24	64	33	0.15
1 wk or longer	43	21	0.10	36	17	0.28
1 mo or longer	15	0	0.08	18	0	0.22
Western Saudi Arabia						
Ever	9	6	0.69	9	8	0.95
1 wk or longer	2	6	0.47	9	8	0.95
1 mo or longer	0	0	-	0	8	1.00
At sea in the Persian Gulf						
Ever	15	5	0.29	27	25	0.90
1 wk or longer	6	0	0.26	9	25	0.31
1 mo or longer	0	0	-	9	25	0.31

* p values indicate probabilities using chi squared test, except when 0 value contained in cell, where Fisher's exact test was used.

Table 10. Association of In-Theater Exposures with Case Status

	Study 2 Cases vs. Controls			Study 1 Variant Cases vs. Study 1 Variant Controls		
	% Cases (n=49)	% Controls (n=19)	p* value	% Cases (n=11)	% Controls (n=12)	p* value
Smoke from oil well fires						
Ever	100	100	1.0	82	67	0.41
1 wk or longer	69	72	0.82	64	33	0.15
1 mo or longer	12	17	0.64	36	8	0.10
Heard chemical alarms sounded						
Ever	79	67	0.29	55	42	0.54
1 wk or longer	21	6	0.14	27	8	0.23
1 mo or longer	6	0	0.23	18	0	0.22
SCUD exploded within 1 mile						
Ever	40	28	0.34	55	27	0.19
1 wk or longer	2	11	0.12	9	0	1.0
1 mo or longer	0	0	-	0	0	-
Engaged in ground combat						
Ever	51	44	0.63	45	25	0.30
1 wk or longer	15	22	0.46	0	8	1.0
1 mo or longer	4	0	0.38	0	0	-
Engaged in air combat						
Ever	6	0	0.56	9	0	0.48
1 wk or longer	2	0	1.0	0	0	-
1 mo or longer	2	0	1.0	0	0	-
Saw U.S. troops killed, wounded						
Ever	35	33	0.92	36	17	0.28
1 wk or longer	0	17	0.02	0	17	0.47
1 mo or longer	0	11	0.07	0	0	-
Saw Iraqis or civilians killed, wounded						
Ever	86	89	0.74	82	58	0.22
1 wk or longer	29	39	0.42	36	25	0.55
1 mo or longer	6	17	0.18	9	0	0.48
Had contact with/handled POWs						
Ever	76	89	0.23	64	50	0.51
1 wk or longer	29	33	0.71	18	8	0.48
1 mo or longer	4	17	0.08	18	0	0.22
Saw/contact with dead animals						
Ever	60	72	0.37	82	42	0.05
1 wk or longer	23	12	0.32	45	0	0.01
1 mo or longer	4	0	1.0	18	0	0.22
Saw destroyed enemy vehicles						
Ever	96	100	1.0	73	50	0.26
1 wk or longer	65	61	0.75	55	33	0.31
1 mo or longer	22	17	0.61	18	8	0.48
Contact w/ destroyed enemy vehicles						
Ever	82	72	0.40	55	42	0.54
1 wk or longer	53	28	0.07	36	8	0.10
1 mo or longer	14	6	0.33	9	8	0.95

	Study 2 Cases vs. Controls			Study 1 Variant Cases vs. Study 1 Variant Controls		
	% Cases (n=49)	% Controls (n=19)	p* value	% Cases (n=11)	% Controls (n=12)	p* value
Contact w/ U.S. friendly fire vehicles						
Ever	27	11	0.18	18	0	0.22
1 wk or longer	2	0	1.0	9	0	0.48
1 mo or longer	2	0	1.0	0	0	-
Used pesticide cream/spray on skin						
Ever	67	67	0.96	55	50	0.83
1 wk or longer	53	44	0.53	55	42	0.54
1 mo or longer	31	6	0.03	36	25	0.55
Wore uniforms treated with pesticides						
Ever	38	17	0.10	36	30	0.76
1 wk or longer	36	11	0.05	36	20	0.41
1 mo or longer	23	0	0.03	18	20	0.92
Wore flea collar						
Ever	4	11	0.28	0	0	-
1 wk or longer	2	6	0.45	0	0	-
1 mo or longer	0	0	-	0	0	-
Living area/camp sprayed with pesticides						
Ever	13	17	0.73	36	36	1.0
1 wk or longer	11	11	1.0	27	9	0.27
1 mo or longer	4	0	1.0	18	9	0.53
Took PB						
Ever	88	83	0.66	91	27	<0.01
1 wk or longer	67	39	0.04	60	18	<0.05
1 mo or longer	21	17	0.70	50	9	0.04
Exposed to CARC paint						
Ever	55	50	0.74	20	18	0.92
1 wk or longer	27	28	0.97	10	18	0.59
1 mo or longer	16	17	0.94	10	9	0.94
Slept < 4 hr in 24 hr period						
Ever	90	83	0.47	91	91	1.0
1 wk or longer	48	61	0.34	55	64	0.66
1 mo or longer	21	17	0.70	9	9	1.0

*p values indicate probabilities using chi squared test, except when 0 value contained in cell, where Fisher's exact test was used.

Table 11. Association of Grouped In-Theater Exposures with Case Status

	Study 2 Cases vs. Controls			Study 1 Variant Cases vs. Study 1 Variant Controls		
	% Cases (n=49)	% Controls (n=19)	p* value	% Cases (n=11)	% Controls (n=12)	p* value
Possible exposure to chemical agents: heard chemical alarms, saw or contact with dead animals						
One or more	87	83	0.68	91	58	0.08
Both	55	56	0.99	45	25	0.30
Poss. exposure to depleted uranium: contact w/ destroyed enemy vehicles, contact w/ friendly fire U.S. vehicles						
One or more	84	72	0.29	55	42	0.54
Both	24	11	0.23	18	0	0.22
Possible traumatic experiences: heard chemical alarms, SCUD within 1 mile, engaged in ground combat, saw U.S. casualties, saw Iraqi or civilian casualties						
1 or more	98	100	1.0	100	67	0.04
2 or more	84	83	0.97	82	58	0.22
3 or more	67	56	0.37	55	17	0.06
4 or more	29	22	0.60	27	8	0.23
All 5	6	0	0.56	9	8	0.95
Possible pesticide exposure: used spray/cream on skin, wore uniform treated with pesticides, wore flea collar, living area sprayed with pesticides						
1 or more	67	72	0.71	64	67	0.89
2 or more	40	28	0.38	45	33	0.58
3 or more	14	6	0.35	18	22	0.82
All 4	2	6	0.52	0	0	-
Possible multiple neurotoxic exposure:						
PB+ poss. chem agent	73	63	0.40	91	25	<0.01
PB+ poss. pesticide	53	58	0.72	64	25	0.06
Poss. chem + poss. pesticide	53	58	0.72	64	33	0.15
PB + chem. + pesticide	47	47	0.97	64	25	0.06

*p values indicate probabilities using chi squared test, except when 0 value contained in cell, where Fisher's exact test was used.

Table 12. Association of Shots/Vaccines with Case Status in Study 2 Case/Control Sample

	Study 2 Cases vs. Controls			Study 2 Volunteers Who Used Shot Records: Cases vs. Controls		
	% Cases (n=49)	% Controls (n=19)	p* value	% Cases (n=10)	% Controls (n=6)	p* value
Received shots not entered in shot record	51	43	0.59	44	67	0.40
Received shot in arm in theater	55	50	0.72	60	67	0.80
Received shot in buttocks in theater	22	6	0.14	22	0	0.34
Gamma globulin shot	95	89	0.34	100	100	-
Typhoid vaccine	92	64	0.02	88	33	0.04
Yellow fever vaccine	77	42	0.02	63	0	0.03
Japanese encephalitis vaccine	25	10	0.32	40	0	0.22
Plague vaccine	65	38	0.11	86	0	0.01
Meningococcus vaccine	40	10	0.08	43	0	0.16
Anthrax vaccine	56	33	0.18	57	40	0.56
Botulinum vaccine	46	45	0.96	43	40	0.92
Used shot record to answer questions	21	32	0.38	100	100	-
Received 5 or more shots (of 8 queried)	33	10	0.16	50	0	0.18
Mean number of shots received (of 8)	3.7	3.0	0.43	4.3	1.8	0.13

Table 13. Association of Shots/Vaccines with Case Status in Variant Sample

	Study 1 Variant Cases vs. Variant Controls		
	% Cases (n=11)	% Controls (n=12)	p* value
Received shots not entered in shot record	82	50	0.14
Received shot in arm in theater	82	62	0.35
Received shot in buttocks in theater	55	30	0.26
Gamma globulin shot	80	89	0.59
Typhoid vaccine	78	90	0.47
Yellow fever vaccine	67	83	0.47
Japanese encephalitis vaccine	50	0	0.10
Plague vaccine	78	83	0.79
Meningococcus vaccine	56	50	0.83
Anthrax vaccine	73	50	0.31
Botulinum vaccine	78	20	0.04
Used shot record to answer questions	27	25	0.90
Received 5 or more shots (of 8queried)	33	40	0.80
Mean number of shots received (of 7)	4.4	4.0	0.72

*p values indicate probabilities using chi squared test, except when 0 value contained in cell, where Fisher's exact test was used.

2.3.4 Association of Case Status With Deployment Locations and Exposures - Multivariate Associations

We further explored the relationships between exposure variables and Case status in logistic regression modeling. The original models tested included all variables found to be associated with increased illness risk at a significance level of 0.10 or less in bivariate analyses. Thus, the original model included age (as a continuous variable), smoking during deployment, being in Bahrain for one week or longer, being in Central Saudi Arabia for one week or longer, contact with destroyed enemy vehicles for one week or longer, using pesticide cream on the skin for one month or longer, wearing a uniform treated with pesticides for one week or longer, and using PB for one week or longer. After controlling for these variables, only age, smoking during deployment, being in Bahrain for one week or longer, having contact with destroyed enemy vehicles for one week or longer, and using pesticide cream on the skin for one month or longer remained significantly associated with illness.

Table 14 provides unadjusted and adjusted odds ratios for variables significantly associated with Case status in multivariable modeling, as well as for wearing uniforms treated with pesticides and using PB for one week or longer. Point estimates for unadjusted odds ratios were quite high for all variables, ranging from 3.14 for PB, to 7.50 for using pesticide cream on the skin. After adjusting for other variables, the point estimates for being in Bahrain longer than one week, having contact with destroyed enemy vehicles, and using pesticide on the skin were substantially higher than for bivariate estimates. After adjustment, estimates for wearing uniforms treated with pesticides and taking PB for longer than one week was somewhat diminished, and was no longer statistically significant.

All analyses of the association of deployment-related exposures to Case status, by definition, involved comparisons only between the 49 Cases and the 19 DC. Given the relatively small sample size for these analyses, especially the small number of Controls, these models yielded relatively unstable estimates of risk. Thus, while significant risk factors observed in the logistic model may, in fact, represent etiologic factors in illness, the magnitude of the association cannot be precisely identified. In addition, point estimates were elevated for a number of other variables, but did not reach statistical significance. Again, it cannot be determined whether the lack of identified risk is merely a reflection of our limited power to detect risk due to sample size, or because those exposures were not actually associated with illness.

Table 14. Multivariate Associations of Location and In-Theater Exposures with Case Status

	% Cases (n=49)	% Controls (n=19)	Bivariate Association (Unadjusted)		Multivariate Association (Adjusted)*	
			Odds Ratio	95% C.I.	Odds Ratio	95% C.I.
Regular smoker during deployment	63%	21%	6.46	1.86 – 22.45	5.04	1.04 – 24.50
In Bahrain ≥ 1 week	60%	28%	3.83	1.17 – 12.53	10.93	1.86 – 64.27
Contact w/destroyed enemy vehicles ≥ 1 week	53%	28%	2.94	0.91 – 9.51	5.51	1.01 – 30.16
Used pesticide on skin ≥ 1 month	31%	6%	7.50	0.92 – 61.63	15.21	1.04 – 222.37
Wore uniforms treated w/pesticide ≥ 1 week	36%	11%	4.53	0.93 – 22.14	3.26	0.42 – 25.13
Took PB ≥ 1 week	67%	4%	3.14	1.02 – 9.65	2.26	0.51 – 10.04

* Maximum likelihood estimates, logistic regression model, adjusted for age, smoking during deployment, being in Bahrain ≥ 1 week, contact with enemy vehicles ≥ 1 week, and using pesticide cream on skin ≥ 1 month

2.3.5 Relationships Between Selected Physiologic Variables, Exposures, and Case Status

For exploratory analyses, logistic models were used to evaluate associations between key physiologic variables (HR, BP, HRV Power, HRV ABS LF, and HRV SDNN at baseline and after up tilt) and Case status (see below). As shown in the unadjusted models in Table 15, Cases were similar to Controls at baseline on all measures except that Cases had higher mean BP. However, after up tilt, the changes in HR, HRV Power, and HRV ABS LF were significantly greater for Controls than for Cases, suggesting a blunted autonomic response in the Cases. These differences remained significant when adding variables representing any use of PB and pesticides to the models (Table 15, Models 1 and 2). However, as expected from the bivariate exposure analyses, these exposure variables were not significantly associated with Case status when added to the logistic models.

Additional models were tested to investigate the association of physiologic variables with use of PB for one week or longer, and pesticides on the skin for one month or longer in predicting Case status, since longer exposure to these substances were associated with Case status in bivariate analyses (Table 15, Models 3 and 4). The associations of Case status with both the physiologic variables and with the exposures remained significant when all were included in the models. Finally, Model 5 in Table 15 models the association of physiologic variables with Case status, controlling for the effects of variables included in the final exposure model, as described previously. While these variables remained significantly associated with Case status in this model, the association of the three physiologic variables with Case status were all diminished. This suggests a possible association between the physiologic differences observed between Gulf War Cases and Controls, and the exposures included in Model 5.

One exception to the pattern described above was seen in the significant association between baseline BP and Case status. In fact, this association was strengthened when any use of PB and pesticides were included in the model (Table 15, Models 1 and 2), suggesting a possible interaction between baseline blood pressure and these two exposures. This phenomenon was not observed when variables for the use of PB or pesticides on the skin for longer time periods were added to the model.

Models were also generated to explore the associations between these same physiologic variables with Cases status among BChE variants, and, overall, with whether the volunteer was a BChE variant or not. As shown in Table 16, only the proportional difference in HRV ABS LF was significantly associated with variant Case status, with that value being higher among Cases than Controls, an opposite effect of that observed in the random Case/Control sample. As discussed previously, there were relatively few variant Cases and Controls, making it difficult to draw reliable statistical conclusions from these results.

Overall, HR at baseline was significantly higher for BChE variant volunteers than for nonvariants, as were HRV Power values following the up tilt. All associations were maintained after controlling for Case status.

Table 15. Logistic Regression Analyses: Study 2 Case/Control Sample Association of Physiological Measures With Case/Control Status

	Mean Values				Cases vs. Controls (p values)						Controls vs. NDC	Cases vs. All Controls
	Cases	Controls	NDC	All Controls	Unadj	Model 1	Model 2	Model 3	Model 4	Model 5	Unadj	Unadj
Mean Heart Rate												
Baseline	69.2	66.5	63.3	64.8	0.314	0.447	0.626	0.244	0.528	0.506	0.294	0.041
Tilt Up 1	81.7	84.7	79.3	81.8	0.302	0.269	0.236	0.481	0.424	0.988	0.157	0.982
Difference: baseline - tilt up 1	12.5	18.2	15.9	17.0	0.009	0.014	0.346	0.015	0.419	0.279	0.374	0.004
Percent difference	18.6	28.5	25.5	26.9	0.011	0.021	0.949	0.014	0.531	0.255	0.506	0.003
Mean Blood Pressure												
Baseline	99.4	93.1	93.6	93.4	0.034	0.043	0.008	0.060	0.423	0.543	0.865	0.013
Tilt Up 1	101.0	96.9	93.5	95.2	0.187	0.215	0.307	0.264	0.203	0.463	0.252	0.021
Difference: baseline - tilt up 1	1.5	3.7	-0.1	1.8	0.370	0.338	0.069	0.389	0.781	0.795	0.181	0.891
Percent difference	1.8	4.4	0.4	2.4	0.325	0.304	0.065	0.365	0.845	0.728	0.195	0.786
HRV Total Power												
Baseline	34.1	36.0	38.8	37.5	0.628	0.973	0.796	0.757	0977	0.500	0.502	0.279
Tilt Up 1	39.0	53.7	49.2	51.3	0.002	0.006	0.013	0.007	0.529	0.148	0.329	0.001
Difference: baseline - tilt up 1	4.9	17.7	10.4	13.8	0.006	0.006	0.011	0.013	0.444	0.053	0.166	0.012
Percent difference	24.6	61.7	42.3	51.3	0.022	0.018	0.034	0.039	0.537	0.059	0.332	0.030
HRV Absolute low frequency power												
Baseline	13.5	15.3	14.3	14.7	0.375	0.502	0.678	0.423	0.800	0.605	0.532	0.485
Tilt Up 1	18.0	26.1	22.6	24.2	0.006	0.015	0.012	0.015	0.469	0.328	0.212	0.003
Difference: baseline - tilt up 1	4.5	10.7	8.3	9.4	0.022	0.027	0.012	0.043	0.356	0.203	0.395	0.016
Percent difference	51.1	87.2	82.7	84.8	0.115	0.121	0.115	0.224	0.389	0.327	0.877	0.074
HRV SDNN												
Baseline	46.4	50.0	59.3	55.0	0.543	0.846	0.391	0.561	0.900	0.619	0.186	0.087
Tilt Up 1	51.6	63.6	63.6	63.6	0.088	0.158	0.686	0.045	0.725	0.384	0.995	0.022
Difference: baseline - tilt up 1	5.2	13.5	4.2	8.5	0.125	0.108	0.075	0.044	0.813	0.101	0.133	0.435
Percent difference	18.7	37.1	16.8	26.2	0.143	0.082	0.202	0.061	0.789	0.080	0.145	0.418

Model 1: Adjusts for any PB, any pesticides
Model 2: Adjusts for any PB, any pesticides, interactions of physiologic variable with PB and pesticides
Model 3: Adjusts for PB > 1 wk, pesticides on skin > 1 mo
Model 4: Adjusts for PB > 1 wk, pesticides on skin > 1 mo, interactions of physiologic variable with PB and pesticides
Model 5: Full exposure model: Adjusts for age, smoking during deployment, in Bahrain > 1 wk, contact with enemy vehicles > 1 wk, pesticides on skin > 1 mo

Table 16. Logistic Regression Analyses: All Volunteers (Includes Variant Sample) Association of Physiological Measures With Variant and Case/Control Status

	Mean Values				Variant Cases vs. Variant Controls (p values)	All Variants vs. All Nonvariants (p values)	
	Variant Cases	Variant Controls	All Variants	All Nonvariants	Unadj	Unadj	Adjusted for c/c*
Mean Heart Rate							
Baseline	62.7	60.7	61.8	67.5	0.515	0.012	0.009
Tilt Up 1	79.7	75.7	77.8	82.1	0.383	0.084	0.082
Difference: baseline - tilt up 1	17.0	15.0	16.0	14.6	0.456	0.361	0.339
Percent difference	26.6	25.4	26.1	22.5	0.766	0.192	0.169
Mean Blood Pressure							
Baseline	95.9	97.1	96.5	96.3	0.774	0.959	0.942
Tilt Up 1	93.4	95.0	93.6	98.0	0.536	0.077	0.059
Difference: baseline - tilt up 1	-3.5	-2.1	-2.9	1.7	0.794	0.059	0.060
Percent difference	-2.7	-1.2	-2.0	2.0	0.773	0.093	0.096
HRV Total Power							
Baseline	32.8	36.9	34.7	35.6	0.454	0.774	0.781
Tilt Up 1	53.1	47.7	50.6	44.2	0.475	0.100	0.086
Difference: baseline - tilt up 1	20.4	10.7	15.9	8.7	0.193	0.060	0.056
Percent difference	76.7	32.7	56.4	35.3	0.074	0.103	0.099
HRV Absolute low frequency							
Baseline	12.7	14.8	13.7	13.9	0.409	0.882	0.889
Tilt Up 1	27.1	23.6	25.5	20.5	0.441	0.033	0.028
Difference: baseline - tilt up 1	14.3	8.8	11.8	6.6	0.230	0.024	0.022
Percent difference	153.9	62.0	111.5	63.2	0.046	0.022	0.022
HRV SDNN							
Baseline	55.4	59.3	57.2	49.6	0.727	0.159	0.148
Tilt Up 1	67.8	63.8	66.0	56.3	0.696	0.086	0.078
Difference: baseline - tilt up 1	12.4	4.5	8.8	6.7	0.391	0.652	0.651
Percent difference	36.9	10.7	24.8	21.6	0.087	0.739	0.739

*Adjusted for Case/Control status

2.4 Results. Study 2: Physiological Studies

Two sets of ANOVAs were performed. One set compared Cases, DC, and NDC; Cases and DC were recruited from the same Army units. The second set compared volunteers who were found to be carriers of genetic variants of BChE with those found to be nonvariants, as defined in our methods section. Unless otherwise noted, the DC and NDC groups were combined, and Case/Control status was included as a variable in the analyses. For some tasks (Deep Breathing, Hand-grip, Mental Arithmetic, Valsalva) the task duration was too short to allow reliable measures of spectral or time domain HRV. To gain some understanding of variability in HR during these tasks, the standard deviation of HR was calculated and analyzed. In both sets of analyses, three major questions were addressed: (1) whether the task produced the expected physiological changes (a main effect for "period"); (2) whether the groups differed from one another (a "group" main effect); and (3) whether the groups differed in reactivity to the task (a "group" by "period" interaction). Statistical trends (effects with $p > .05$ but less than .10) are also reported to provide additional information to other investigators.

2.4.1 Case Group Analysis

(1). Did performance of the ATB tasks produce the expected physiological changes? Tables 17 and 18 summarize the physiological effects of each of the tasks listed in the order in which they were performed in the battery. For tasks that had more than one part, the means and standard deviations for each part are shown. For example, Deep Breathing had three parts, baseline, Trial 1 and Trial 2. While the direction of change for Deep Breathing was as expected (reduced mean HR and mean BP), the effect was not statistically significant. Examination of the tables indicates that for all other tasks, highly significant results in the expected direction were obtained, verifying the valid implementation of the tasks and of the measurement procedures.

Table 17. Physiological Effects (Mean, SD) of the Autonomic Test Battery

Task	Variable	F	df	p <	Baseline	Trial 1	Trial 2
Deep Breathing	Mean HR	3.07	2, 176	.07	68.6(9.6)	67.8(8.8)	68(9.0)
	Mean BP	2.88	2, 176	.08	91.6(10.9)	89.5(8.6)	89.9(8.9)
	SBP			ns			
	DBP			ns			
Hand-grip	Mean HR	88.39	2, 174	.0001	68.6(9.7)	70.3(9.8)	75.2(10.7)
	SD HR	8.44	2, 174	.0004	2.6(1.6)	2.9(1.2)	2.8(1.4)
	Mean BP	101.32	2, 174	.0001	91.5(10.9)	98.2(10.2)	107.7(14.7)

Task	Variable	F	df	p <	Baseline	Trial 1	Trial 2
	SBP	90.57	2, 174	.0001	127.2(13.5)	134(13.5)	143.5(18.2)
	DBP	83.82	2, 174	.0001	72.8(10.1)	78.9(9.4)	86.7(13.2)
Arithmetic	Mean HR	86.64	1, 85	.0001	67.3(9.8)	73.2(10.2)	
	SD HR	27.19	1, 85	.0001	2.9(1.4)	3.6(1.4)	
	Mean BP	49.49	1, 85	.0001	91.5(9.6)	98.5(11.2)	
	SBP	43.49	1, 85	.0001	125.7(12.8)	134.2(14.8)	
	DBP	45.39	1, 85	.0001	73.3(9.1)	79.4(10.1)	
Valsalva	Mean HR	16.55	2, 170	.0001	68.7(9.7)	72.2(9.8)	70.9(9.6)
	SD HR	266.2	2, 170	.0001	2.5(1.4)	9.0(4.4)	9.0(4.5)
	Mean BP	49.88	2, 170	.0001	91.7(11.1)	100.2(9.3)	98.7(8.9)
	SBP	48.95	2, 170	.0001	127.5(13.6)	136.7(13.8)	134.4(12.5
	DBP	73.75	2, 170	.0001	72.9(10.2)	83(8.3)	81.5(8.3)
Emotional	Mean HR	185.8	1, 88	.0001	67.3(9.9)	73.2(10.5)	
	Mean BP	20.70	1, 88	.0001	94.6(9.6)	98.1(10.3)	
	SBP	22.50	1, 88	.0001	129.9(13.1)	134.3(13.5)	
	DBP	13.36	1, 88	.0004	76(8.8)	78.6(8.9)	
	Power	36.41	1, 88	.0001	28.7(12.4)	34.9(13.4)	
	ABS LF	16.62	1, 88	.0001	11.2(5.2)	13.1(5.4)	
	ABS HF			ns			
	L/H ratio	21.05	1, 88	.0001	1.15(.5)	1.36(.5)	
	%LF	4.61	1, 88	.035	39.4(7.7)	37.6(6.8)	
	%HF	58.77	1, 88	.0001	37.7(9.5)	30.3(8.9)	
	SDNN	32.98	1, 88	.0001	48.7(24.8)	61.4(30.1)	
	RMSSD	4.70	1, 88	.03	40.8(32.3)	37.3(37.8)	
	SDSD	4.66	1, 88	.03	40.9(32.4)	37.4(37.9)	

Task	Variable	F	df	p <	Baseline	Trial 1	Trial 2
	%NN			ns			
Initial Up-Tilt	Mean HR	462.0	1, 86	.0001	67.2(10.1)	81.7(10.9)	
	Mean BP	2.95	1, 71	.09	96.3(10)	98.0(10.4)	
	SBP			ns			
	DBP	33.11	1, 71	.0001	78.2(9.2)	83.9(9.2)	
	Power	33.19	1, 86	.0001	35.7(14.6)	44.7(15.9)	
	ABS LF	57.91	1, 86	.0001	14.1(7)	20.9(9.4)	
	ABS HF	12.64	1, 86	.0006	13.3(7.4)	10.7(5.9)	
	L/H ratio	140.2	1, 86	.0001	1.22(.5)	2.15(.8)	
	%LF	40.39	1, 86	.0001	39.2(7.7)	45.7(8.8)	
	%HF	158.3	1, 86	.0001	36(9.7)	23.4(7.3)	
	SDNN	11.90	1, 86	.0009	50.4(23.3)	57.1(23.7)	
	RMSSD	63.65	1, 86	.0001	39.7(29.6)	23.5(16)	
	SDSD	63.75	1, 86	.0001	39.8(29.6)	23.5(16.0)	
	%NN	46.26	1, 86	.0001	15.2(18.1)	5.3(9.4)	
Initial Down-Tilt	Mean HR	11.47	1, 84	.001	67.3(10.2)	69.4(9.6)	
	Mean BP	18.80	1, 74	.0001	95.3(10.2)	89.5(12.1)	
	SBP			ns			
	DBP	24.30	1, 74	.0001	76.9(9.9)	70.6(11.2)	
	Power	19.01	1, 84	.0001	35.7(14.7)	28.7(11.5)	
	ABS LF	19.58	1, 84	.0001	14.0(7.1)	10.8(4.3)	
	ABS HF	26.87	1, 84	.0001	13.4(7.5)	9.7(6.4)	
	L/H Ratio	3.87	1, 84	.06	1.2(.5)	1.4(.7)	
	%LF			ns			

Task	Variable	F	df	p <	Baseline	Trial 1	Trial 2
	%HF	7.25	1, 84	.009	36.2(9.7)	32.6(11.8)	
	SDNN	332.0	1, 84	.0001	50.3(23.5)	111.9(47.7)	
	RMSSD	43.69	1, 84	.0001	40.0(29.8)	66.6(64.3)	
	SDSD	43.9	1, 84	.0001	40.1(29.9)	66.9(64.9)	
	%NN	43.6	1, 84	.0001	15.4(18.2)	21.2(17.5)	

Table 18. Physiological Effects of 80° Head Up-Tilt and Recovery From Tilt

Variable	Baseline	Min 0-5 Up	Min 6-10 Up	Min 11-15 Up	Min 16-20 Up	Initial Down	Min 2-5	Min 6-10
Mean HR	67.5(10.2)	81.6(11.1)	84(11.5)	85.3(11.6)	86.9(11.8)	69.4(9.6)	63(9.8)	64.0(9.5)
Mean BP	96.1(10.2)	97.9(10.8)	93.3(8.2)	93(9)	93.6(8)	89.5(12.2)	92.9(8.9)	93.2(9.6)
SBP	129.3(12.5)	127.8(13.4)	124.2(11.2)	123.5(11.2)	125(9.8)	NS		
DBP	78.2(9.3)	83.9(10.5)	79(7.8)	79(8.6)	79.3(7.8)	70.6(11.3)	73.8(8.3)	74.7(8.9)
Power	35.7(15.3)	44.4(15.6)	38.2(15.3)	38.7(18.4)	38.3(16.9)	28.7(11.5)	28.4(16.5)	33.6(14.8)
ABS LF	14.6(7.3)	21.1(9.6)	18.1(9.1)	18.4(9.6)	18(9.2)	10.8(4.3)	10.8(5.7)	13.1(7.0)
ABS HF	12.8(7.4)	10.3(5.3)	9.5(5)	10(6.6)	10.3(6.5)	9.7(6.4)	10.7(9.6)	12.2(6.6)
L/H Ratio	1.28(.5)	2.2(.8)	2.1(.8)	2.1(.7)	2.0(.7)	1.4(.7)	1.2(.6)	1.2(.5)
%LF	40.4(7.2)	46.5(8.8)	46.4(8.6)	47.6(9.2)	46.8(8.7)	NS		
%HF	34.6(9)	22.7(6.7)	24.6(7.5)	25(7.3)	25.7(8.1)	32.6(11.8)	36.2(10.6)	35.9(9.9)
SDNN	49.8(24.2)	56.6(23.8)	44.2(19.9)	45(21.2)	42.4(19.3)	111.9(47.7)	64.9(62.3)	59.5(26.8)
RMSSD	38.0(30.3)	23(14.9)	21.9(15.2	22(15.7)	22.1(17.7)	66.6(64.3)	55.8(90.4)	48.2(31.3)
SDSD	38.1(30.4)	23(14.9)	21.9(15.2)	22(15.7)	22.1(17.7)	66.9(64.9)	56(90.7)	48.3(31.3)
%NN	13.4(17.0)	5.5(9.9)	5(9.7)	4.9(9)	4.1(7.1)	21.2(17.5)	20.7(19.6)	21(19.9)

(2). Did the groups differ in their physiological responses during performance of the ATB? The DC and NDC groups were compared; if they did not differ ($p > .10$) the two Control groups were combined and compared to the Cases. Table 19 summarizes the results. Although the maximum sample size was 49 for Cases and 42 for Controls, the actual sample size for various tasks and variables differed. For example, three veterans did not participate in the head-up tilt test because they showed PVCs during the initial baseline, and 13 veterans were returned to the horizontal position before the end of the 20-min head-up tilt period because they showed signs of syncope. To use as much data as possible, the first 5-min period of head-up tilt was analyzed for all 89 veterans who completed the initial portion of the test; the 75 veterans with complete data were included in an analysis of all the 5-min head-up tilt periods. Since equipment problems occurred most frequently for measures of tonometric BP, sample size was smaller for BP variables than for those based on HR.

Table 19. Physiological Differences Between Cases and Combined Control Groups

Variable	Task	Cases (Mean, SD)	Controls (Mean, SD)	F	df	p<
Mean BP	Baseline	93.5 (11.2)	89.1 (8.7)	4.34	1, 89	.04
	Deep Breathing	92.6 (9.6)	87.7 (8.8)	8.39	1, 89	.005
	Emotional Stress	98.4 (10.5)	93.9 (9)	5.63	1, 89	.02
	Tilt Baseline	99.4 (10)	93.4 (9.2)	7.32	1. 72	.009
	Tilt Up, 5 min	100.2 (10.5)	94.3 (9.1)	8.19	1, 72	.006
	Tilt Up, 20 min	97.4 (9.6)	91.2 (7.9)	10.46	1, 52	.002
SBP	Tilt Up, 20 min	129.0 (12.3)	121.8 (9.8)	8.01	1, 52	.007
DBP	Deep Breathing	73.9 (9.1)	70.3 (8.0)	5.25	1, 89	.03
	Emotional Stress	79 (9.7)	75.3 (7.6)	4.65	1, 89	.04
	Tilt Baseline	81 (8.9)	75.5 (8.8)	6.98	1, 72	.01
	Tilt Up, 5 min	83.7 (10.1)	78.5 (9.2)	7.43	1, 72	.008
	Tilt Up, 20 min	82.2 (9.1)	76.8 (8)	8.51	1, 52	.005
	Tilt Down, 10 min	75.9 (10.4)	72 (9)	4.66	1, 74	.04
Mean HR	Emotional Stress	72.2 (10.8)	67.8 (9.8)	4.58	1, 89	.04

Variable	Task	Cases (Mean, SD)	Controls (Mean, SD)	F	df	p<
	Tilt Down, 1 min	70.4 (10.1)	66 (9.2)	4.99	1, 85	.001
Spectral HRV						
Total Power	Tilt Up, 5 min	35.6 (15.2)	44.4 (15.7)	9.65	1, 87	.003
	Last Up v First Down	29.6 (12.1)	39.1 (17.6)	16.91	1, 85	.0001
ABS LF	Tilt Up, 5 min	15.8 (8.9)	19.5 (8.6)	9.80	1, 87	.003
	Tilt Up, 20 min	15.6 (7.8)	21.1 (9.8)	13.88	1, 76	.004
	Tilt Down, 1 min	11.6 (6.7)	13.3 (5.1)	4.78	1, 85	.04
	Tilt Down, 10 min	11.63 (7.2)	12.8 (5)	4.07	1, 85	.05
	Last Up v First Down	12.02 (5.5)	17.4 (9.5)	25.36	1, 85	.0001
%LF	Tilt Up, 20 min	43.9 (9.1)	47.5 (8.2)	7.09	1, 76	.01
ABS HF	Tilt Up, 5 min	10.6 (5.9)	13.6 (7.5)	7.08	1, 87	.009
	Last Up v First Down	9.0 (6)	11.8 (7.1)	7.23	1, 85	.009
Time-Domain HRV						
SDNN	Tilt Baseline	46.4 (23.6)	55.0 (22.2)	4.66	1, 87	.04
	Tilt Up, 5 min	49.0 (25.1)	59.3 (20.8)	8.43	1, 87	.005
	Tilt Up, 20 min	43.4 (22.6)	52.8 (20.8)	7.08	1, 76	.01

Variable	Task	Cases (Mean, SD)	Controls (Mean, SD)	F	df	p<
	Tilt Down, 1 min	73.2 (41.6)	89.9 (54.2)	6.32	1, 85	.02
	Tilt Down, 10 min	64.1 (36.9)	80.1 (58.9)	6.94	1, 85	.01
	Last Up v First Down	69.4 (43.1)	88.2 (55.1)	9.65	1, 85	.003
rMSSD	Tilt Down, 1 min	44.5 (45.3)	63.2 (56.8)	7.58	1, 85	.007
	Tilt Down, 10 min	44.0 (38.1)	62.4 (76.6)	5.59	1, 85	.02
SDSD	Tilt Down, 1 min	44.7 (45.6)	63.4 (57.2)	7.58	1, 85	.007
	Tilt Down, 10 min	44.1 (38.4)	62.6 (77)	5.60	1, 85	.02
	Last Up v First Down	36.7 (45.4)	54.1 (58.3)	5.49	1, 85	.03
%NN	Tilt Baseline	12.0 (16.4)	19 (19)	6.85	1, 87	.01
	Tilt Up, 5 min	8.1 (14.1)	12.8 (16.2)	8.90	1, 87	.004
	Tilt Up, 20 min	5.5 (11.5)	7.9 (11.5)	5.82	1, 76	.02
	Tilt Down, 5 min	14.7 (16.8)	22.4 (18.6)	5.74	1, 85	.02
	Last Up v First Down	10.3 (14.5)	15.7 (16.6)	5.92	1, 85	.02

While all of the ATB tasks produced significant alterations in physiology in the expected direction, these did not serve to differentiate between Case and Control groups during the Valsalva, hand-grip and mental arithmetic tasks. The initial, 5-min baseline was included to determine whether the groups differed after coming to the laboratory environment, and being fitted with measuring devices. Cases had higher mean BP than Controls. Mean BP was also higher for Controls during Deep Breathing, Emotional Stress, the Tilt Baseline, and both the initial and total periods of head-up tilt. DBP was also higher for Cases than Controls during the Deep Breathing, Emotional Stress, and Tilt tasks. Mean HR was greater for Cases during Emotional Stress, and when, at the end of head-up tilt, the veteran was returned to the horizontal position.

The head-up tilt test resulted in more group differences than any of the other ATB tasks, and these differences were particularly pronounced for HRV measures. The initial 5-min tilt-up period revealed less spectral Power, ABS LF power, and ABS HF power in the Cases than in the Controls. ABS LF power and %LF power were less for Cases throughout the 20-min period of tilt. When the last tilt-up period was compared with the first period after return to the horizontal position, Power, ABS LF, and ABS HF were lower for Cases than for Controls.

Time domain measures of HRV yielded similar results. During the initial tilt-up period, SDNN and %NN were lower for Cases, and this difference was maintained throughout the tilt-up test. Returning to the horizontal position was also associated with lower SDNN, rMSSD and SDSD for Cases than Controls. When the last tilt-up period was compared with the first period of recovery, SDNN, SDSD and %NN were again lower for Cases.

For some of the tasks and variables included in the ATB, significant differences were found between the DC and NDC groups. Table 20 summarizes these results. During the initial baseline, the NDC group exhibited a higher %HF than either the DC or Case groups. DBP during the Mental Arithmetic task was less for the NDC group. During the first 5 min of head-up tilt, rMSSD and SDSD were greater for the NDC group than for either of the other groups. Statistical trends ($.05<p<0.10$) were found for L/H and %HF. L/H was less for the NDC group than for the other two groups, while %HF was greater for the NDC group than for the other two groups. The HRV results indicate a greater degree of parasympathetic variability in NDC than in those who were deployed.

Table 20. Results for Task/Variable Combinations Where Control Groups Could Not Be Combined

Task	Variable	DC vs. NDC, p <	Cases (Mean, SD)	DC (Mean, SD)	NDC (Mean, SD)	F	df	p =
Baseline	%HF	.06	36.0 (8.3)	36.9 (9.2)	43.3 (11.7)	4.80	2, 88	.02
Arithmetic	DBP	.05	77.7 (10.2)	77.7 (11.4)	72.4 (7.4)	3.22	2, 88	.05
Tilt up, 5 min	L/H	.03	1.7 (.08)	1.9 (.08)	1.5 (.08)	2.54	2, 86	.09
Tilt up, 5 min	%HF	.04	29.0 (10.2)	28.0 (9.7)	32.6 (11.8)	2.66	2, 86	.08
Tilt up, 5 min	rMSSD	.07	27.5 (22.7)	28.7 (15.9)	43.1 (32.4)	5.12	2, 86	.008
Tilt up, 5 min	SDSD	.07	27.6 (22.8)	28.7 (15.9)	43.1 (32.5)	5.12	2, 86	.008

To provide more detailed information for other investigators undertaking similar research, we have included Table 21, which shows the means and standard deviations, together with statistical results, for all tasks and variables that approached significance.

Table 21. Case/Control Differences in Physiological Response (Mean, SD) to the ATB

Task	Variable	F	df	p<	Cases	DC	NDC
Baseline	Mean BP	2.46	2, 88	<.10	93.5(11.2)	90.4(9.2)	88(8.4)*
	L/H ratio	3.38	2, 88	.04	1.16(.4)	1.15(.5)	0.94(.4)*
	%HF	4.80	2, 88	.02	36(8.3)	36.9(9.2)	43.3(11.7)*
	RMSSD	2.53	2, 88	.09	30.2(20.5)	30.6(20.1)	55.5(59.3)*
	SDSD	2.53	2, 88	.09	30.2(20.6)	30.6(20.1)	55.6(59.4)*
Deep Breathing	Mean BP	4.21	2, 88	.02	92.6(9.6)	88.2(8.4)*	87.4(9.2)*
	DBP	2.86	2, 88	.06	73.9(9.1)	71.2(8.0)	69.5(8.0)*
Arithmetic	DBP	3.05	2, 85	.06	77.7(10.2)	77.7(11.4)	72.4(7.4)*
Emotional Stress	Mean HR	2.84	2, 88	.07	72.2(10.8)	69.6(10.0)	66.4(9.5)*
	Mean BP	3.37	2, 88	.04	98.4(10.5)	95.5(10.5)*	92.6(7.4)*
	DBP	3.22	2, 88	.05	79(9.7)	77.1(8.7)	73.9(6.3)*
	%HF	2.96	2, 88	.06	32.6(8.9)	32.9(7.4)	37.9(12.5)*
	RMSSD	2.98	2, 88	.06	33.9(24.5)	34.4(21.6)	53.8(54.6)*
	SDSD	2.97	2, 88	.06	34(24.6)	34.5(21.7)	53.9(54.8)*
	%NN	2.80	2, 88	.07	11.9(15.2)	11.9(14.3)	20.4(20)*
Initial Up-Tilt	Mean BP	4.17	2, 71	.02	100.2(10.5)	95(9.7)	93.6(8.5)*

Task	Variable	F	df	p<	Cases	DC	NDC
	DBP	4.12	2, 71	.02	83.7(10.1)	79.7(9.9)	77.3(8.3)*
	Power	4.80	2, 86	.02	36.6(15.1)	44.8(15.9)*	44.0(15.7)*
	ABS LF	5.30	2, 86	.007	15.8(8.9)	20.7(9.6)	18.4(7.7)
	ABS HF	3.97	2, 86	.03	10.6(5.9)	12.1(5.4)*	14.8(8.8)*
	L/H ratio	2.54	2, 86	.09	1.7(.8)	1.9(.8)	1.5(.8)*
	%HF	2.66	2, 86	.08	29(10.2)	28(9.7)	32.7(11.8)*
	SDNN	4.38	2, 86	.02	49(25.1)	56.8(19.7)	61.4(21.7)*
	RMSSD	5.12	2, 86	.008	27.5(22.7)	28.7(15.9)	43.1(32.4)*
	SDSD	5.12	2, 86	.008	27.6(22.8)	28.7(15.9)	43.1(32.5)*
	%NN	5.22	2, 86	.007	8.1(14.0)	8.8(10.9)	16.2(19.1)*
20 min Up-Tilt	Mean BP	5.46	2, 51	.007	97.4(9.6)	92.1(8.6)*	89.8(6.5)*
	SBP	3.93	2, 51	.03	129.0(12.3)	121.8(10)	121.9(9.6)
	DBP	4.64	2, 51	.01	82.2(9.1)	77.8(8.8)	75.2(6.3)*
	Power	5.56	2, 75	.006	35.2(15)	45.6(16.7)*	41.8(17.4)
	ABS LF	7.71	2, 75	.001	15.6(7.8)	22.5(10.2)*	19.5(9.3)*
	%LF	3.98	2, 75	.03	43.9(9.1)	48.4(7.9)*	46.5(8.4)
	SDNN	3.50	2, 75	.04	43.4(22.6)	51.8(19.6)*	53.7(22.1)*
	%NN	3.08	2, 75	.06	5.5(11.5)	6.2(8.8)	9.7(13.6)*
Initial Down-Tilt	Mean HR	3.05	2, 84	.06	70.4(10.1)	67.5(9.3)*	64.6(8.9)*
	ABS LF	2.43	2, 84	.10	11.6(6.7)	13.6(5.0)	13.0(5.3)

Task	Variable	F	df	p<	Cases	DC	NDC
	SD NN	3.30	2, 84	.05	73.2(41.6)	90.1(63.7)*	89.7(45.2)*
	RMSSD	4.07	2, 84	.03	44.5(45.3)	61.4(69.1)	64.7(44.4)*
	SDSD	4.07	2, 84	.03	44.7(45.6)	61.7(69.6)	65(44.7)*
	%NN	3.59	2, 84	.04	14.7(16.8)	18.6(17)	25.7(19.4)*
10 Min Down	Mean HR	3.01	2, 84	.06	68(10.3)	65.4(9.5)*	62.1(8.8)*
	DBP	2.46	2, 73	.10	75.9(10.4)	72.8(8.6)	71.4(9.3)*
	ABS LF	2.41	2, 84	.10	11.6(7.2)	13.5(5.2)*	12.2(4.8)*
	SDNN	3.43	2, 84	.04	64.1(36.9)	83.4(75.5)*	77.1(39.4)*
	RMSSD	2.80	2, 84	.07	44.0(38.1)	64.5(103.4)	60.5(42.2)*
	SDSD	2.80	2, 84	.07	44.1(38.4)	64.8(103.8)	60.7(42.4)*
	%NN	3.30	2, 84	.05	15.9(18.0)	21.5(17.1)	25.6(20.5)*
Prepulse Inhibition	Startle	6.25	2, 84	.003	330(370)	611(464)*	639(552)*
	Prepulse	6.78	2, 84	.002	12.1(31.3)	22.7(40.3)	65.8(104.6)*
	PPI score	4.70	2, 76	.012	6.7(24.2)	3.4(6.3)	15.4(30.3)*

* Pairwise comparison showed that the marked Group differed from the Case Group at $p < .05$

During the initial resting baseline, Cases had higher L/H ratio and lower %HF than the NDC, but did not differ from the DC group, implying less variability in the vagal output for veterans who had been deployed to the Persian Gulf. During Deep Breathing, Cases had higher mean BP than Controls, but this appears to be a continuation of the group differences observed at baseline. During the Emotional Stress task, mean HR, mean BP and DBP were higher for Cases than for the NDC group. No difference between Cases and DC were found for either spectral or time domain HRV measures; Cases did, however, have lower HRV than the NDC group. The groups did not differ in their ratings of the vividness with which they recalled the stressful incident; Cases, however, rated the stressfulness of the incident higher than either DC or NDC ($F(2, 88) = 5.58$, $p = .005$).

Three veterans showed PVCs during the initial baseline, and according to pre-established criteria, were not allowed to participate in the subsequent tilt task. An additional 13 volunteers had to be lowered before the end of the 20-min tilt up period because they showed signs of syncope. To use as much available data as possible, the first 5-min period of "up-tilt" was evaluated for all 112 volunteers in the tilt test; the 99 with complete data were included in an analysis of all of the 5-min tilt up periods. Most of the missing data were BP data, thus the sample size for BP variables was smaller than that for HR.

Analysis of the data for the 99 veterans who completed the entire task indicated that during the first five min of the head-up tilt, mean BP and DBP were higher for Cases than for NDC. All time-domain HRV measures were lower for Cases than for NDC. These findings are consistent with the results of the spectral HRV measures. Over the entire 20 min of head-up tilt, mean BP and SBP were lower for Cases than for DC; mean BP and DBP were also lower for Cases than for NDC. Cases had lower HRV than DC or NDC using both spectral and time domain measures. When volunteers were returned to the horizontal position, Cases also had lower time-domain HRV than NDC and higher mean HR than either Control group. These group differences tended to persist throughout the recovery period. The missing data from the 16 volunteers who did not complete the task were randomly distributed across the three groups. A number of the observations obtained from head-up tilt are illustrated in the following Figures.

Pre-pulse inhibition (PPI) is the reduction in the startle response that is produced by a brief auditory warning pulse. As is traditional, we divided the EMG response to the auditory pulse by the EMG response to the startle stimulus, and multiplied the result by 100 to derive the PPI Score. The higher the score the greater the inhibition. NDC showed greater inhibition than Cases; the difference between Cases and DC was not significant. We also performed ANOVA on the absolute response to the startle stimulus and the absolute response to the auditory pulse. Cases had a smaller startle response than either Control group ($F\ 2, 84) = 6.25$, $p = .003$). Response to the auditory pulse was also smaller for Cases compared to the NDC group; the pairwise comparison between Cases and DC was not significant.

(3). Did the groups differ in reactivity? Only the up-tilt and tilt recovery segments of the battery revealed significant differences in reactivity. Table 22 shows the means and standard deviations for each component of the task for each of the three groups. During the first 5 min of up-tilt, Cases showed less HR reactivity than the other groups (see Figure 5). This same blunted reactivity was found for

mean HR when only veterans with complete data were included in the analysis, and all four, 5-min periods of up-tilt were examined (see Figure 6).

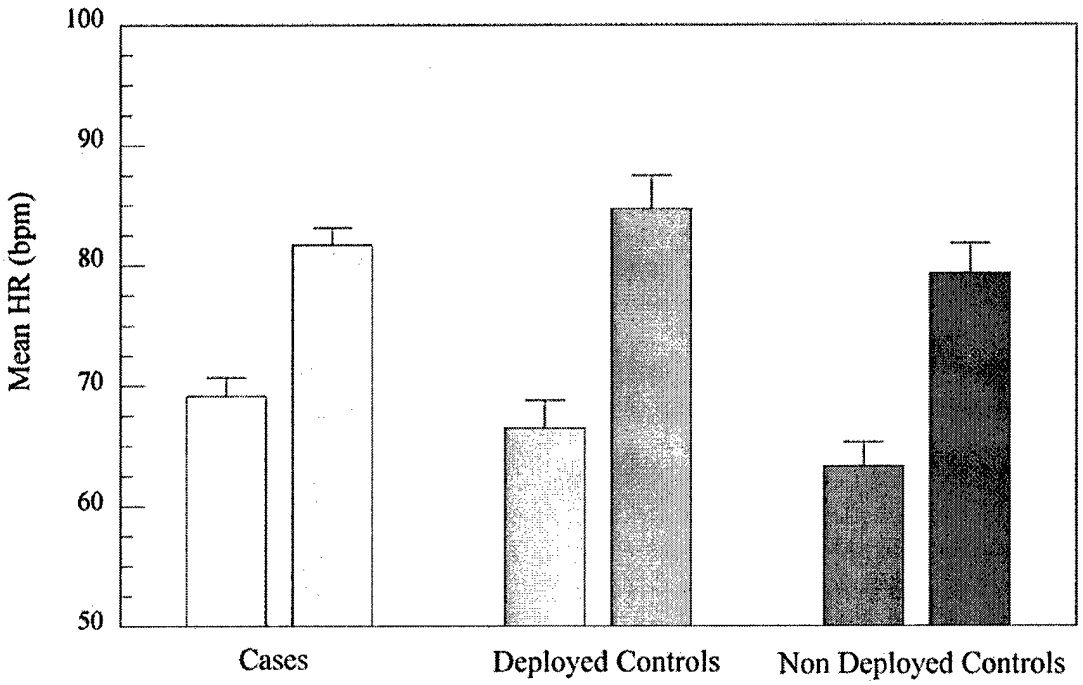


Figure 5. Changes in HR from the tilt baseline to the initial 5-min period of head up-tilt. Cases showed less reactivity to the task than either Control group; $F(2,86) = 6.16$, $p = 0.003$. For each group, baseline is indicated by the left bar.

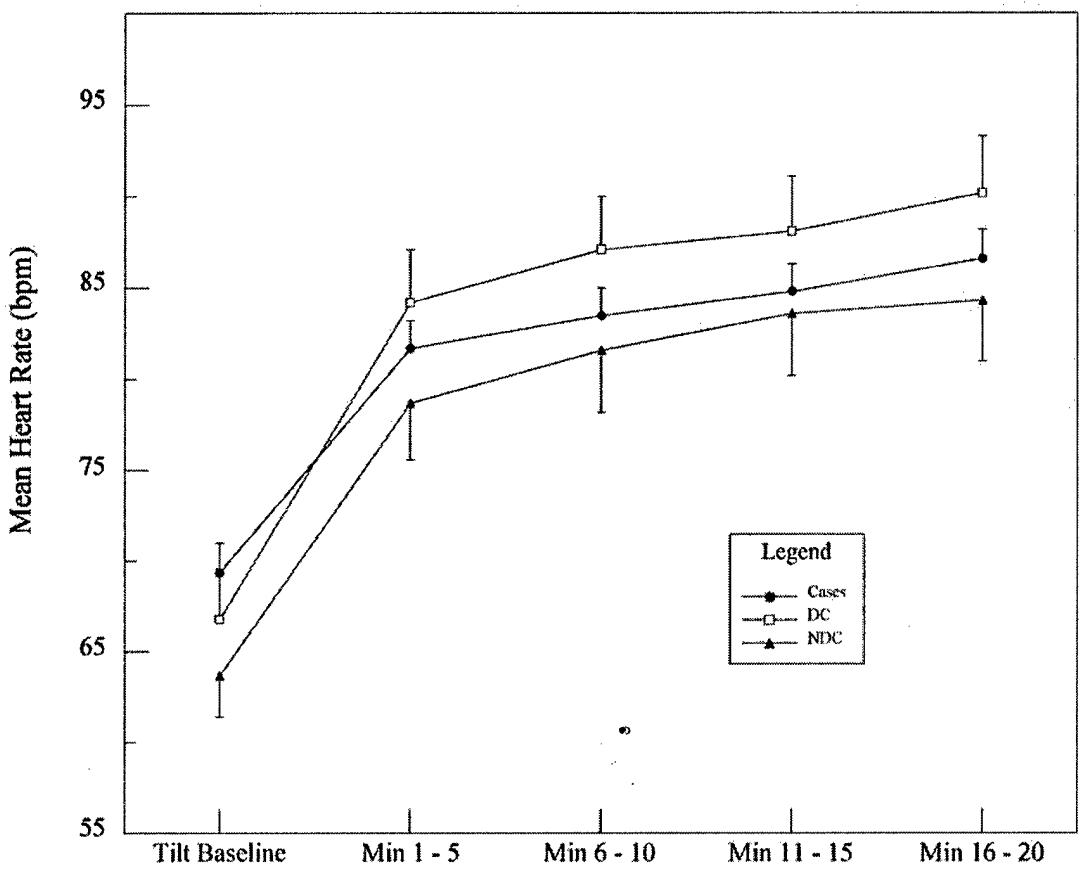


Figure 6. Heart rate over the 20-min head up-tilt. Cases showed less initial increase in HR than either Control group, and less elevation of HR throughout the task; F(8,300) = 2.94, p = 0.006.

Immediately after being returned to the horizontal position, both Cases and NDC showed decreases in SBP compared to the baseline immediately before head-up tilt, while DC volunteers showed increased SBP. There was also a trend for %LF power to differ among the groups; Cases did not show a %LF power response to down-tilt (38 vs. 38), while DC showed a decrease (42 vs. 38) and NDC showed an increase (38 vs. 41). No significant differences in time-domain HRV were found. We also examined recovery from tilt using the last 5 min of head-up tilt and the first period after returning to the horizontal position. The two Control groups did not differ from one another. Cases had a lower ABS LF than Controls ($F_{1,57} = 8.03$, $p = .006$), and this interacted with period ($F_{1,57} = 4.80$, $p = .033$). The Cases showed a smaller decrease in ABS LF (14.4 to 10) than the DC group (22.6 to 11.6). No interaction between group and period was found when the Case group was compared to the NDC group, although Cases had lower ABS LF than NDC. These effects may be due to lower Power for the Case group than either of the two Control groups ($F_{2,73} = 4.22$, $p < .02$), an interpretation supported by the lack of group differences or group by period interactions of %LF. Time-domain measures of HRV did not

show differences in reactivity when recovery from tilt was analyzed using the last 5 min of head-up tilt as the starting point.

When all three periods of tilt recovery were examined, a group difference in reactivity was found for SBP and is shown in Figure 7. Cases showed an initial small decrease in SBP, but this decrease quickly returned to baseline levels. The DC group, on the other hand, initially showed in increase in SBP. The NDC group exhibited a pattern similar to that seen for the Case group, although the effect did not reach the .05 level of significance. Reactivity as measured by time-domain HRV was significant for rMSSD, SDSD and %NN. For all of these measures, the change from baseline to the first period of down tilt was greater for the DC than for the Case group, and the change lasted longer for DC than for the other two groups. No such differences were found for spectral HRV variables.

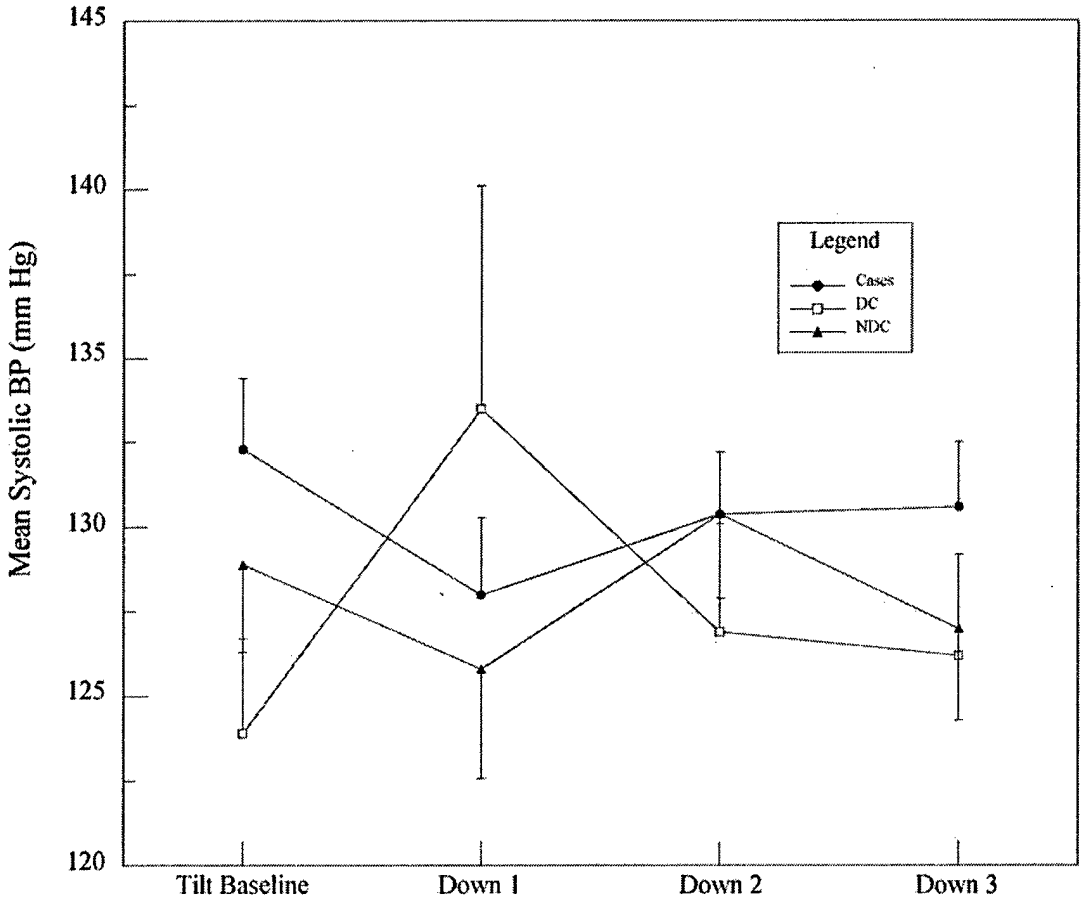


Figure 7. Changes in SBP on returning to the horizontal position after 20 minutes of head-up tilt. The pattern for the DC group differs from the Cases and the NDC. $F(6,219) = 2.92$, $p = 0.009$.

Table 22. Case/Control Differences in Physiological Response (Mean, SD) to 80° Head-Up Tilt and Recovery from Tilt

Variable	Group	Baseline	Min 0-5	Min 6-10	Min 11-15	Min 16-20	Down Min 1	Min 2-5	Min 5-10
Mean HR	Cases	69.4(10.2)	81.7(9.9)	83.5(9.9)	84.8(9.8)	86.6(10.3)	NS		
	DC	66.8(10.1)	84.2(12.3)	87.1(12.4)	88.1(12.8)	90.2(13.1)			
	NDC	63.7(9.3)	78.7(12.7)	81.6(13.9)	83.6(14.2)	84.2(13.7)			
SBP	Cases	132.3(12.8)	NS				128.0(14.5)	130.4(11.5)	130.6(11.8)
	DC	123.9(11.6)					133.5(27)	126.9(13.0)	126.2(12.3)
	NDC	128.9(11.6)					125.8(14.5)	130.4(11.0)	127.0(12.2)
rMSSD	Cases	34.7(27.3)	NS				54.3(56.6)	40.2(28.0)	46.8(31.0)
	DC	34.4(16.5)					88.4(89.5)	92.2(181.8)	43.1(18.1)
	NDC	56(38.2)					73.5(49.2)	56.9(41.2)	55.6(39.6)
SDSD	Cases	34.8(27.4)	NS				54.5(57.1)	40.3(28.1)	46.9(31.1)
	DC	34.5(16.5)					88.9(90.1)	92.5(182.5)	43.2(18.1)
	NDC	56.1(38.2)					73.9(49.6)	57.1(41.3)	55.8(39.7)
%NN	Cases	12.3(16.7)	NS				17.0(16.8)	16.2(18.7)	18.1(19.7)
	DC	12.5(12.7)					24.8(18.7)	26.5(17.0)	22.4(17.2)
	NDC	24.6(22.5)					26.9(16.1)	25.0(21.9)	26.0(22.1)

2.4.2 Variant Group Analysis

Table 23 summarizes the group differences in physiological responses found between genetic variant and nonvariant veterans. Only those task/variable combinations that were statistically significant, or approached significance ($p <= 0.10$) are listed. Across all tasks, the variant group had lower mean HR than the nonvariant group, and there were trends for the variant group to also have lower BP. During baseline, more group differences were found for spectral than for time-domain measures of HRV, while during tasks, more group differences were found for time-domain HRV.

Table 23. Variant/Nonvariant differences (Mean, SD) during the ATB

Task	Variable	F	df	p <	Variant	Nonvariant
Baseline	Mean HR	5.99	1, 110	.02	64.2(7.6)	69.1(9.6)
	Power	3.04	1, 110	.09	31.8(13.2)	27.5(13.6)
	ABS LF	7.72	1, 110	.006	13.2(4.9)	10.4(5.0)
	L/H ratio	4.39	1, 110	.04	1.4(.7)	1.1(.4)
	%LF	8.75	1, 110	.004	42.3(8.2)	38(6.2)
	SDNN	7.32	1, 110	.008	53.6(29.4)	39.9(25.5)
Breathing	Mean HR	6.74	1, 110	.02	63.5(7.1)	68.4(9.1)
	DBP	2.84	1, 110	.10	69.5(7.8)	72.3(8.8)
Hand-grip	Mean HR	6.65	1, 109	.02	66.4(8.7)	71.6(10.4)
Arithmetic	Mean HR	4.36	1, 107	.04	66.4(10.1)	70.6(10.3)
Valsalva	Mean HR	7.30	1, 106	.008	65.7(7.3)	71(9.7)
Emotional	Mean HR	6.74	1, 110	.02	65.3(8.9)	70.6(10.4)
	DBP	2.75	1, 110	.10	74.5(7.4)	77.4(9.1)
	Power	4.32	1, 110	.04	36.7(14.7)	31.5(13.2)
	ABS LF	4.09	1, 110	.05	13.9(5.4)	12(5.3)
	SDNN	12.46	1, 110	.0006	73.7(35)	54.2(28)
	rMSSD	6.05	1, 110	.02	56(49.7)	38(34.8)
	SDSD	6.05	1, 110	.02	56.1(49.8)	38.1(34.9)

Task	Variable	F	df	p <	Variant	Nonvariant
Initial Up-Tilt	Mean HR	5.44	1, 107	.02	69.8(12.7)	74.8(12.7)
	DBP	3.34	1, 92	.07	77.9(10.3)	81((10)
	SDNN	3.28	1, 107	.07	61.6(28)	53(22.8)
	RMSSD	3.06	1, 85	.09	35.7(28.2)	26.8(18.8)
	SDSD	3.06	1, 85	.09	35.7(28.2)	26.9(18.8)
20 min Up-Tilt	Mean HR	3.62	1, 95	.06	76.8(13.8)	81.5(13)
	%NN	3.18	1, 95	.08	10.1(14.5)	6.1(10.7)
Initial Down-Tilt	Mean HR	4.76	1, 105	.03	64.4(8.7)	68.6(9.8)
	SDNN	3.43	1, 105	.07	92(50.7)	80.6(48.6)
Recovery	Mean HR	5.91	1, 105	.02	61.6(9.1)	66.2(9.9)
	DBP	4.04	1, 94	.05	70.6(10.2)	74.2(9.9)
Prepulse Inhibition	Startle	7.65	1, 106	.007	738(599)	475(469)
	Pre-pulse	3.02	1, 106	.09	68.3(118.7)	29.4(64.7)
	PPI Score	4.30	1, 78	.04	11.8(19.9)	5.9(20.4)

To understand differences between the variant and nonvariant groups in reactivity to the tasks, 3-way ANOVAs were performed for each task; the ANOVAs included variant status, Case status, and period. Differences in reactivity to the tasks between the variant and the nonvariant groups are shown in Tables 23 and 24. Again, only those effects that reached or approached statistical significance are included in the table. The variant group showed more reactivity to Mental Arithmetic (SBP, $p < .05$), Emotional Stress (Power, $p < .08$), head-up tilt (Mean BP, $p < .06$; ABS LF power $p < .06$), and recovery from tilt (HR, $p < .02$; mean BP, $p < .03$; SBP, $p < .07$). The nonvariant group was more reactive to head-up tilt (DBP $p < .04$) and recovery from tilt (Power, $p < .06$).

When recovery from tilt was examined using the more immediate last 5 min of head-up tilt rather than the baseline before tilt as the starting point, variant volunteers were more reactive than nonvariant volunteers as measured by DBP ($F\ 1,65 = 5.11$, $p < .03$, see Figure 8) and by mean BP ($p < .06$, see Figure 9). There was a variant group by Case group by period interaction for %NN ($F\ (1,93) = 5.57$, $p = .02$) and trends for variant group by Case group interactions by period interactions for SDNN ($p < .07$), RMSD ($p < .10$) and SDSD ($p < .10$). For SDNN and %NN, there was little difference between the variant Cases and variant Controls, while the nonvariant Cases had lower values than the nonvariant Controls. For RMSSD and SDSD, Cases had lower values than Controls, and this difference was much larger for the nonvariant compared to the variant volunteers. The effect on mean HR of returning to the

horizontal position was no longer significant when the last period of head-up tilt was used as the baseline.

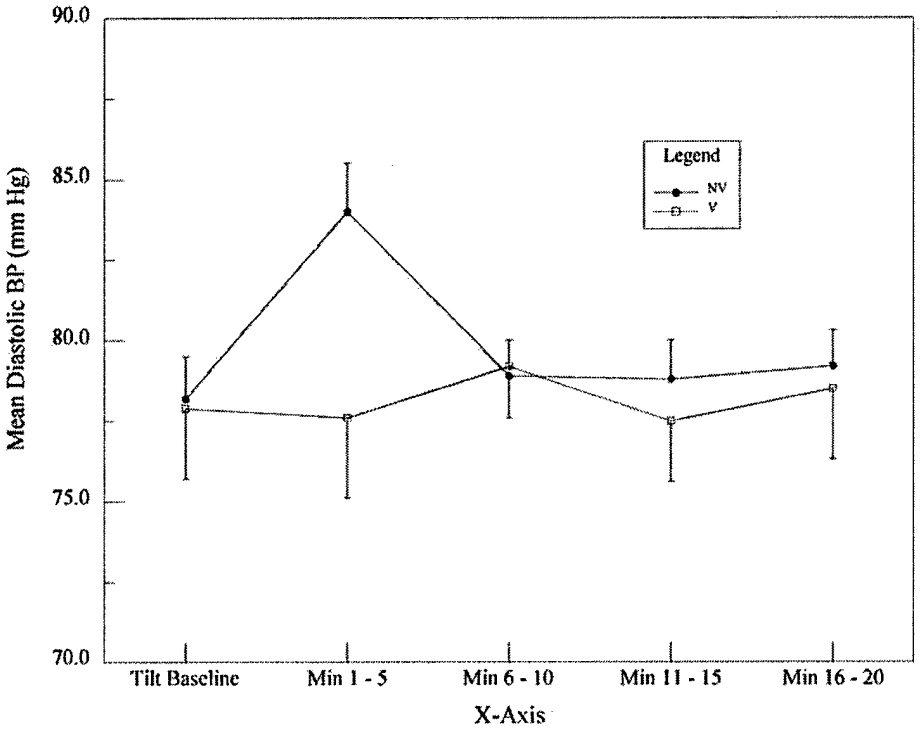


Figure 8. Changes in mean DBP during 20-min head-up tilt. Nonvariant volunteers showed an initial increase in DBP upon tilt, which variant volunteers did not; during minutes 6-20 the group differences were no longer observed. $F(4,272) = 2.68$; $p < 0.04$.

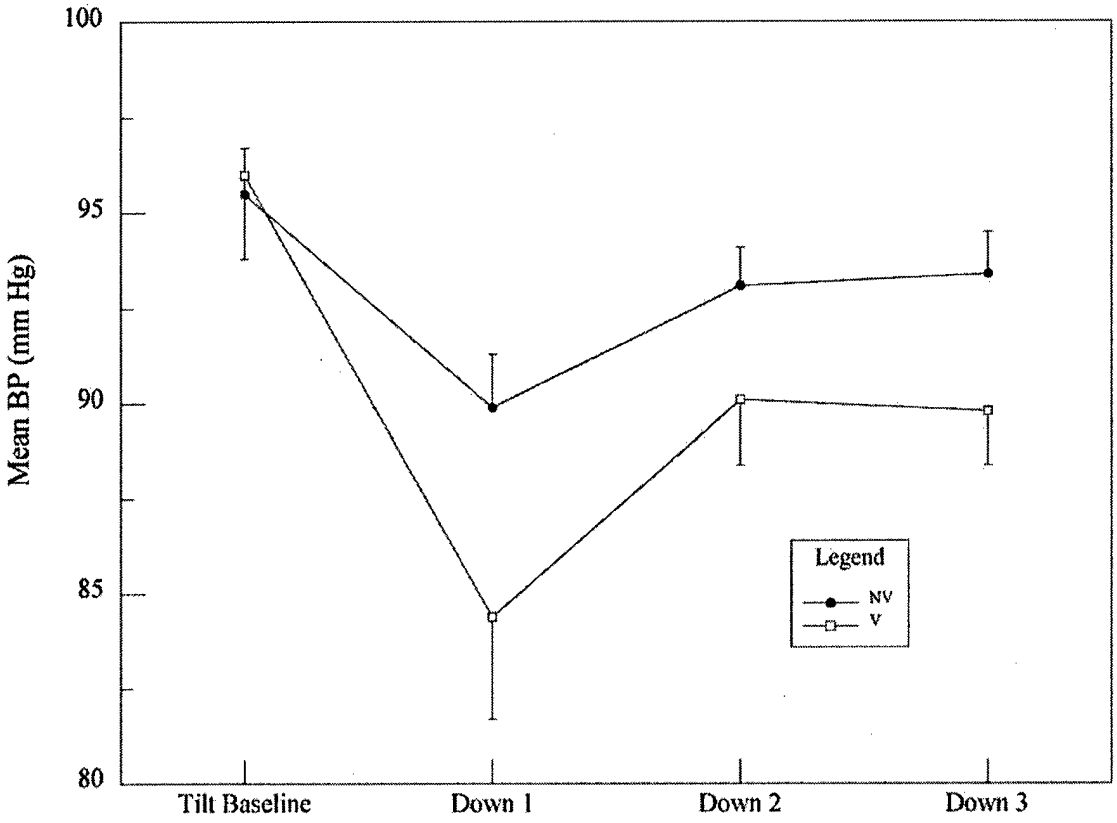


Figure 9. Variant volunteers, compared to nonvariants, showed a trend for lower mean BP when returned to the horizontal position after 20-min of head-up tilt. $F(3,282) = 2.61$, $p = 0.06$.

The variant analysis revealed an interaction between variant status, Case status, and grip strength ($F(1,105) = 4.50$, $p < .04$). Grip strength was greater for the dominant hand than the nondominant hand except for variant Cases, for whom there was no difference (53.8 v. 53.5). Variants also rated the vividness of the recall of a stressful event lower than the nonvariants ($F(1,110) = 4.10$, $p < .05$). Nonvariant Cases rated the stressfulness of the event higher than nonvariant Controls, but no such difference was found between variant Cases and Controls ($F(1,110) = 3.97$, $p < .05$).

The PPI score, the amplitude of the startle response, and the amplitude of the response to the pre-pulse were all higher for the variant group than for the nonvariant group; no interactions between variant group and Case/Control group were found.

Table 24. Variant/Nonvariant Differences in Physiological Response (Mean, SD) to the ATB

Task	Variable	F	df	p	Group	Baseline	Task
Arithmetic	SBP	4.15	1, 107	.04	Var	123.7(10.5)	137.4(13.6)
					NonVar	125.9(13)	134.3(14.7)
Valsalva*	SD HR	2.74	2, 212	.08	Var	2.7(1.2)	7.6(4.3)
					NonVar	2.5(1.4)	9.1(4.4)
Emotional	Power	3.15	1, 110	.08	Var	30.7(12.8)	42.7(14.2)
					NonVar	28.4(12.4)	34.6(13.3)
Initial Up Tilt	Mean BP	3.79	1, 92	.06	Var	96.5(10.9)	93.6(11.1)
					NonVar	96.4(10)	98(10.5)
	DBP	4.02	1, 92	.05	Var	77.4(9.5)	78.4(11.1)
					NonVar	78.2(9.3)	83.9(9.9)
	ABS LF	3.78	1, 107	.06	Var	13.7(6.3)	25.5(11.7)
					NonVar	13.9(6.8)	20.5(9.5)
20 min Up Tilt	Mean BP	2.44	4, 272	.06	Var	See Table 25	
					Non Var	See Table 25	
	DBP	2.68	4, 272	.04	Var	See Table 25 and also Fig 4	
					NonVar	See Table 25 and also Fig 4	
Initial Down Tilt	Mean HR	6.70	1, 105	.01	Var	61.8(7.9)	67(8.8)
					NonVar	67.6(10.1)	69.6(9.5)
	Mean BP	4.85	1, 95	.03	Var	96(10.8)	84.4(13.5)

Task	Variable	F	df	p	Group	Baseline	Task
					NonVar	95.5(10.3)	89.9(12)
	SBP	3.57	1, 74	.06	Var	131(13.5)	125.4(17.2)
					NonVar	130.3(13.1)	130.6(19.1)
	DBP	5.01	1, 95	.03	Var	76.8(9.4)	65.0(13.0)
					NonVar	77.0(10)	70.9(11.2)
	Power	3.74	1, 105	.06	Var	34.7(14.2)	32.9(11.3)
					NonVar	35.6(14.4)	28.5(11.8)
Recovery	Mean HR	4.53	3, 315	.01	Var	See Table 25	
					NonVar	See Table 25	
	Mean BP	2.61	3, 282	.06	Var	See Table 25	
					NonVar	See Table 25	
	SBP	2.33	3, 222	.10	Var	See Table 25	
					NonVar	See Table 25	
	DBP	2.66	3, 282	.06	Var	See Table 25	
					NonVar	See Table 25	
	Power	2.18	3, 315	.09	Var	See Table 25	
					NonVar	See Table 25	

*Note: Only Valsalva 1 data is shown in table.

Table 25. Variant/Nonvariant Differences in Physiological Response (Mean, SD) to 80° Head-up Tilt and Recovery from Tilt

Variable	Group	Baseline	Min 0-5	Min 6-10	Min 11-15	Min 16-20	Initial Down	Min 2-5	Min 5-10
Mean HR	Var	61.8(7.9)					67.0(8.8)	58.2(8.7)	59.5(8.8)
	NonVar	67.6(10.1)					69.6(9.5)	63.3(9.7)	64.2(9.3)
Mean BP	Var	97.2(11.7)	93(11.7)	94.3(7.8)	93.3(8.5)	94.2(10.5)	84.4(13.5)	90.1(8.3)	89.8(7.0)
	NonVar	96.1(10.3)	98(11)	93.2(8.4)	92.9(8.8)	93.6(8)	89.9(12.1)	93.1(8.7)	93.4(9.7)
SBP	Var	131.0(13.5)					125(17.2)	129(11.9)	128(9.6)
	NonVar	130.3(13.0)					131(19.1)	130(11.7)	130(12.0)
DBP	Var	77.9(10.2)	77.6(11.7)	79.2(7.7)	77.5(9.1)	78.5(10.4)			
	NonVar	78.2(9.4)	84(10.5)	78.9(8)	78.8(8.5)	79.2(7.8)			
l Power	Var	34.7(14.2)					32.9(11.3)	26.0(11.8)	34.9(15.8)
	NonVar	35.6(14.4)					28.5(11.7)	28.3(16.9)	33.4(15.0)

In addition to reactivity differences between Cases, DC and NDC, and between variants and nonvariants, interactions between the two grouping factors were found for several of the tasks. During the Mental Arithmetic task DBP measures from the DC and NDC groups were statistically different, and it was not possible to combine them; we therefore dropped the NDC from the Mental Arithmetic analysis for DBP. Variant status by Case/Control status by period interactions were found for mean BP ($F_{1,107} = 8.58$, $p < .004$), SBP ($F_{1,107} = 4.21$, $p < .05$), and DBP ($F_{1,85} = 9.26$, $p = .003$). Figure 10 summarizes the results for DBP; mean BP showed the same pattern. The variant Controls were most reactive to the task, and the variant Cases were least reactive. For SBP, variant Controls were the most reactive and nonvariant Controls were the least reactive.

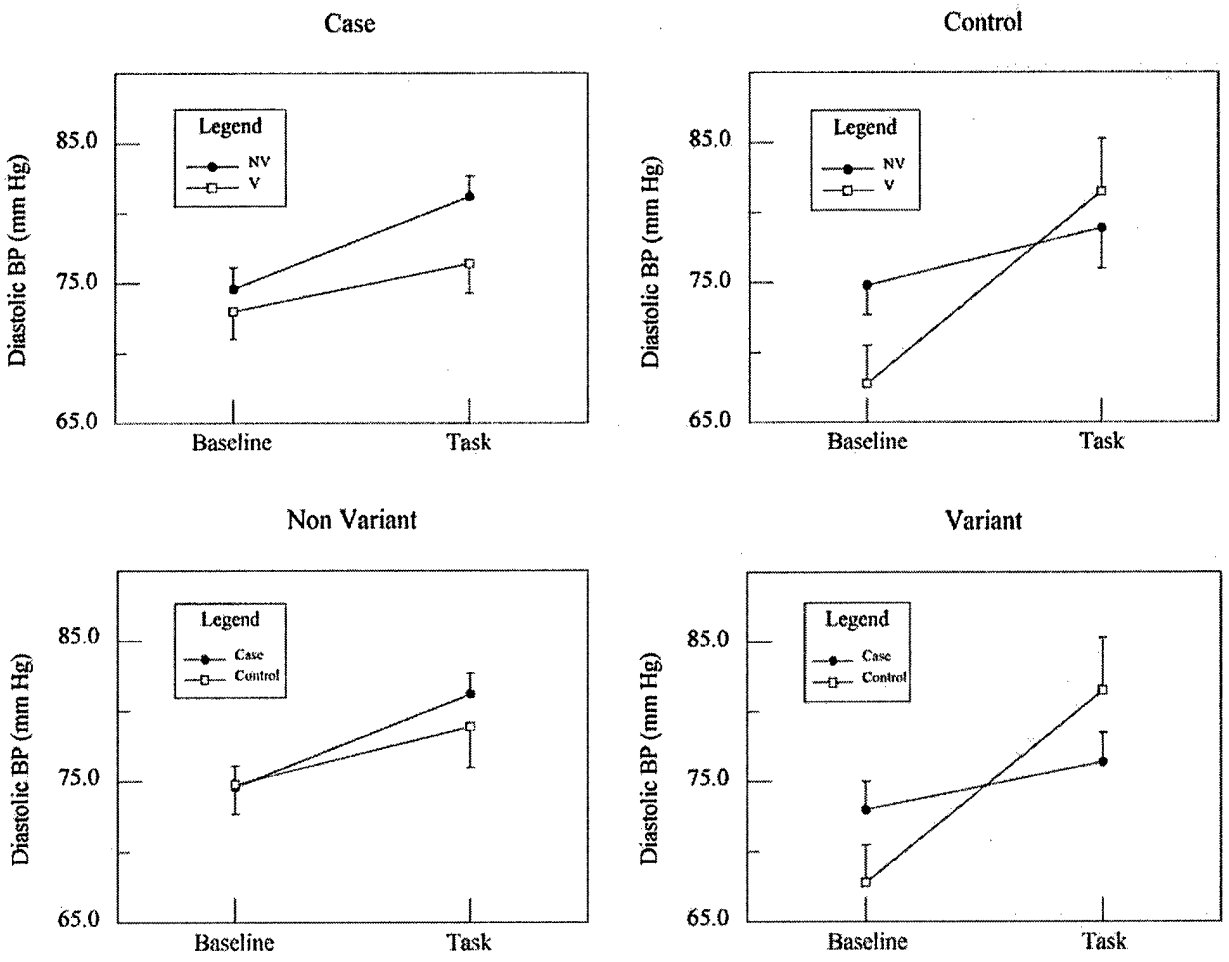


Figure 10. Changes in DBP as a function of performing the Mental Arithmetic task were affected by both Case status and genetic variant status ($F_{1,85} = 9.26$, $p = 0.003$). The two upper boxes show the interaction by Case vs. Control; the two lower boxes show the same interaction, focusing on Variant vs. Nonvariant status.

For the Emotional Stress task, only %HF showed a significant variant group by Case group by period interaction ($F\ 1,110 = 4.63$, $p < .04$). As shown in Table 26, performing the task reduced %HF for all variant by Case groups, but this decrease was greatest for variant Cases.

Table 26. Variant/Nonvariant Differences in Percent High Frequency HRV (%HF) During the Emotional Stress Task

Group	Baseline Mean (SD)	Task Mean (SD)
Nonvariant Cases	36(7.8)	29(8.1)
Nonvariant Controls	40(10.5)	32(9.5)
Variant Cases	40(8.7)	28(7.3)
Variant Controls	37(15.2)	33(10.9)

The Mean HR response to head-up tilt differed as a function of both Case/Control group and variant group ($F\ 1,107 = 4.82$, $p = .03$); the interaction is shown in Figure 11. Nonvariant Cases started with higher HR than the other three groups, but showed less responsivity to tilt. Time-domain HRV, as measured by SDNN, showed a trend ($p = .06$) for an interaction between variant group, Case group and period. Variant Cases showed a greater increase in SDNN upon head-up tilt than the other three groups. Cases also had lower %NN than Controls ($F\ (1,107) = 7.14$, $p = .009$); this difference was due to the nonvariant volunteers (variant group by Case group interaction $F\ (1,107) = 5.04$, $p < .03$); the nonvariant Case group had significantly lower %NN than the nonvariant Control group (7.1 vs. 13.0). The variant Cases and Controls did not differ. Significant interactions were also found for spectral HRV measures. An interaction between Case group, variant group and period ($F\ (1,107) = 8.97$, $p = .003$) was found for total spectral power; tilt increased power for all variant/Case combinations, and this increase was smallest for the nonvariant Cases (34 v 38), and greatest for the variant Cases (33 vs. 53). A similar pattern was seen for power in the ABS LF band ($F\ (1,107) = 10.03$, $p = .002$). During the initial 5 min of head up-tilt, nonvariant Cases had lower ABS LF power than nonvariant Controls, while Cases and variant Controls did not differ ($F\ (1,95) = 4.58$, $p < .04$). As shown in Figure 12, an interaction between Case group, variant group and period was also found for ABS LF power ($F\ (4,380) = 3.24$, $p = .02$) over the full period of head up-tilt. While Variant Cases showed the greatest reactivity, Nonvariant Cases showed less initial reactivity to tilt than the other groups. When volunteers were returned to the horizontal position, no interactions between Case group and variant group were found, nor were there significant differences in reactivity.

In summary, interactions between variant status, case status and period indicated that variant cases were less reactive than variant controls during mental arithmetic (DBP), and more reactive to emotional stress (%HF) and head-up tilt (SDNN, ABS LF). Nonvariant cases were less reactive than nonvariant controls during head-up tilt when HR was the variable of interest, but more reactive when ABS LF was measured.

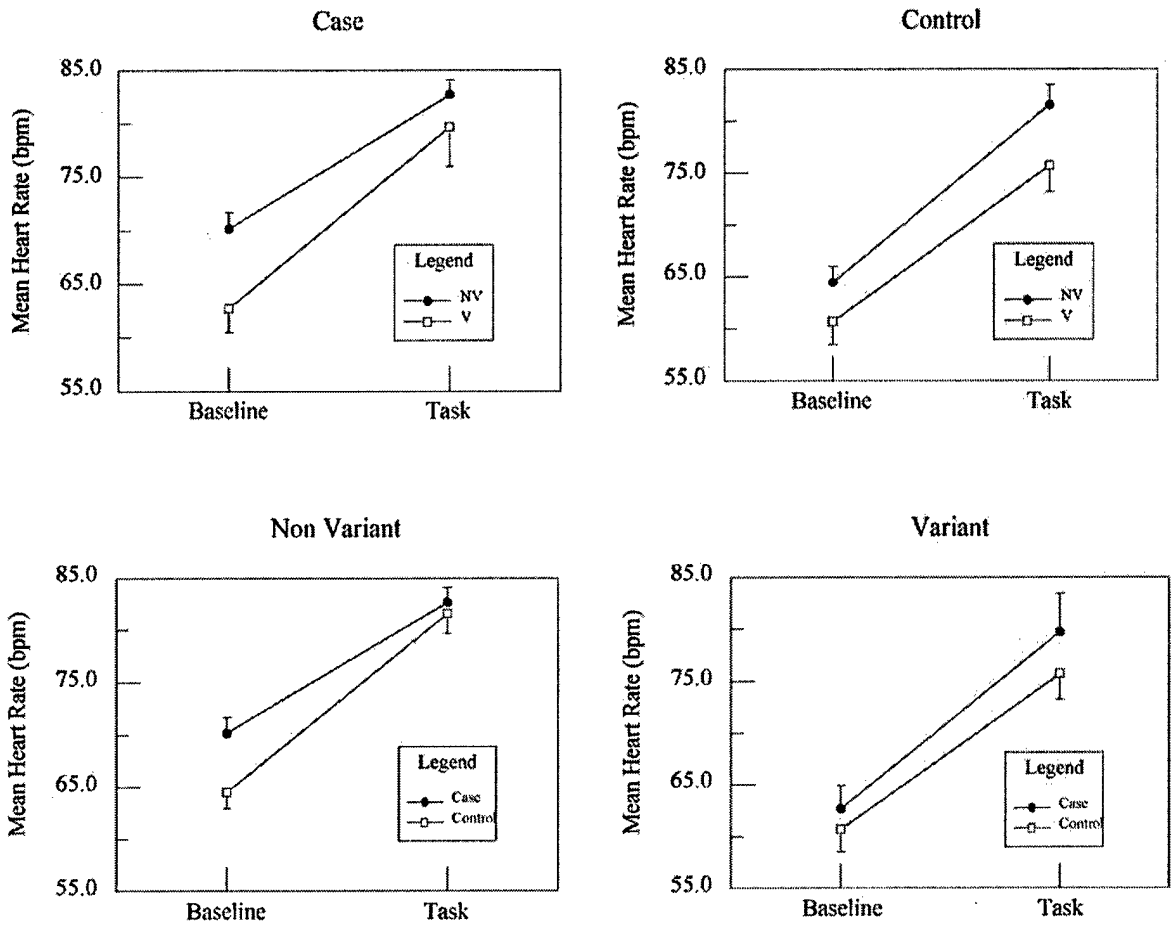


Figure 11. Changes in the HR response to head-up tilt over the first five min were affected by both Case status and Variant status (F (1,107) = 4.82, p = 0.03). The interaction is shown in two ways. The two upper boxes focus on Cases vs. Controls, while the lower boxes focus on Variant status.

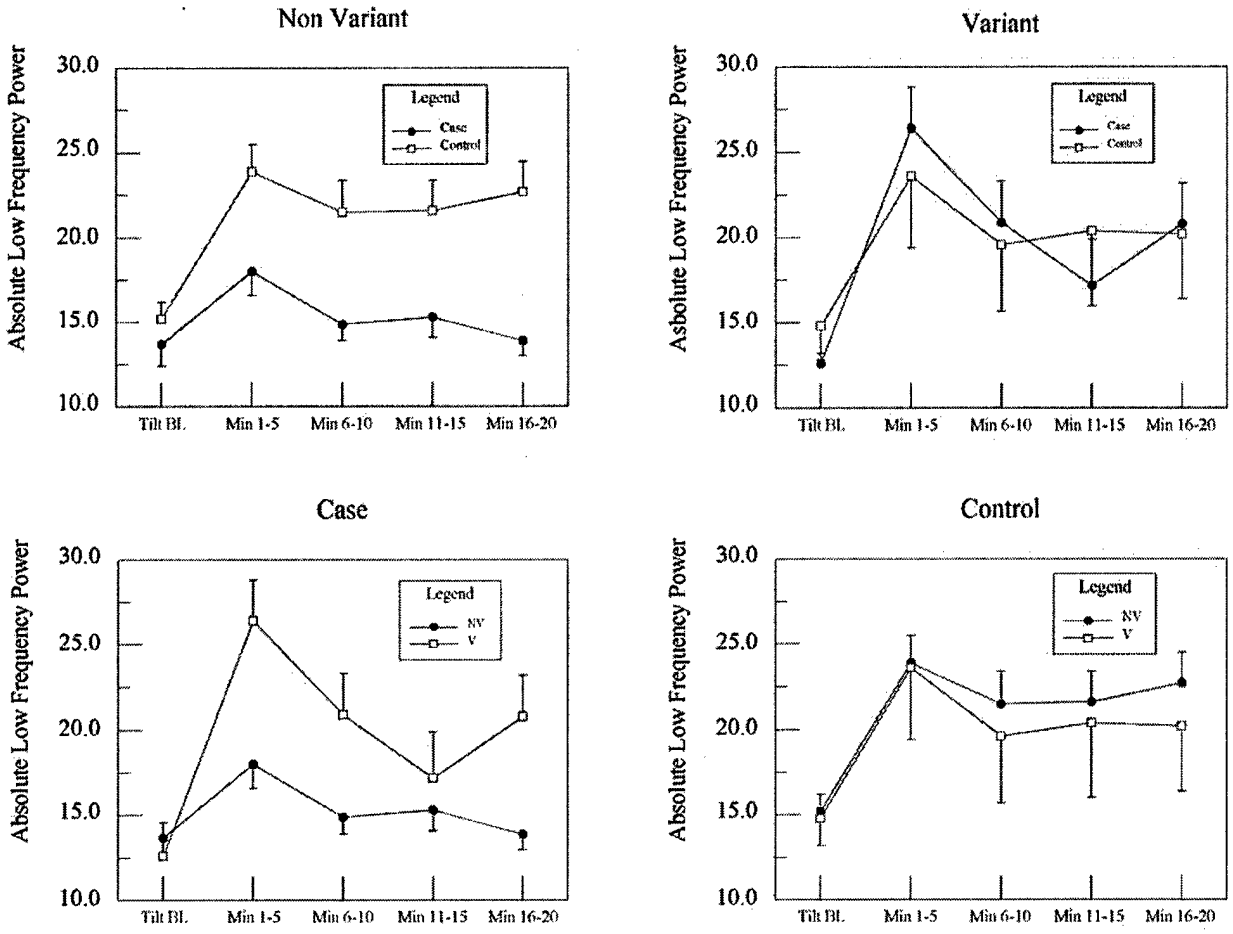


Figure 12. The HRV response to head up-tilt, as measured by LF Power, was altered by both Case status and Variant status, $F(4,380) = 3.24$, $p < 0.02$. The top panels focus on Cases vs. Controls, while the bottom panels illustrate Variants vs. Nonvariants.

2.5 Results. Supplementary Studies.

2.5.1 Epidemiology and Exposure Assessment.

Validity of Veterans' Self-Reported Exposures. Attempts to evaluate, in epidemiologic studies, the extent to which Gulf War-related exposures may have contributed to the development of GWI have principally relied on veterans' reports concerning exposures they encountered during deployment. Self-reported exposures are generally considered to be unreliable owing to concerns about the accuracy of veterans' recall and their inability, in some Cases, to know whether or not they were exposed to specific substances such as depleted uranium, low-level nerve agents, or individual vaccines. Because of these types of concerns, our questionnaire was designed to elicit veterans' reports of their

experiences in theater, as opposed to what they were exposed to. For example, we did not ask veterans whether they had been exposed to depleted uranium, but asked instead whether they had been near or come into direct contact with destroyed enemy vehicles. Similarly, we did not ask veterans if they were exposed to chemical weapons, but whether they had the experience of hearing chemical alarms sound while they were in theater. In addition, we did not ask veterans if they had received the anthrax or botulinum toxoid vaccines, but rather asked if they had received shots in the arm during deployment, since the anthrax and botulinum vaccines were the only ones routinely delivered in theater.

In this supplementary task, we performed a series of evaluations to determine whether self reported exposures "made sense" and exhibited internal consistency when viewed in the context of known facts about the Gulf War. For example, only individuals who were present in theater at certain time periods and in certain locations should have reported exposure to oil well fire smoke. Assessment of the degree to which self-reported exposures by veterans appeared to be logically consistent with known facts about the war can provide a general indicator of whether reports of exposures in theater are likely to be, in the best Case, reasonably informative for identifying associations with GWI, or in the worst Case, too fraught with recall and/or reporting bias to provide useful information.

Tables 27 and 28 summarize exposures reported in Study 1 by Gulf War veterans who served in different locations and time periods during deployment.

Table 27. Proportion of Gulf War Veterans in Study 1 Reporting Individual Exposures, by Veterans' Reported Location in Theater

	Not In Iraq/Kuwait			**In Iraq and/or Kuwait** (n=177)	Comments
% of veterans reporting exposure to:	At sea ≥ 1 wk (n=32)	E. Saudi ≥ 1 wk (n=56)	On land, other (n=39)		
Smoke from oil well fires	44	70	33	88	Reasonable: highest in Kuwait, E. Saudi; lower at sea, elsewhere
Heard chemical alarms	29	75	30	60	Reasonable: highest in Kuwait, E. Saudi; lower at sea, elsewhere
SCUD exploded within 1 mile	10	52	32	42	Unknown
Directly involved in ground combat	0	0	0	49	Reasonable: only reported if in Iraq/Kuwait
Directly involved in air combat	3	0	8	7	Reasonable: rarely reported, only in Iraq/Kuwait, ship, distant support areas
Saw U.S./allied troops killed/wounded	12	29	24	45	Reasonable: Highest in battlefield areas, lower in support areas
Saw Iraqis/civilians killed/wounded	6	23	8	79	Reasonable: Highest in battlefield areas, lower in support areas
Had contact with prisoners of war	6	30	8	67	Reasonable: Highest in battlefield areas, lower in support areas
Saw or had contact with dead animals	9	29	16	60	Reasonable: Highest in battlefield areas, lower in support areas
Saw destroyed enemy vehicles	16	29	16	97	Reasonable: Highest in battlefield areas, lower in support areas
Had contact with destroyed enemy	3	16	3	75	Reasonable: Highest in battlefield

% of veterans reporting exposure to:	Not In Iraq/Kuwait			In Iraq and/or Kuwait (n=177)	Comments
	At sea ≥ 1 wk (n=32)	E. Saudi ≥ 1 wk (n=56)	On land, other (n=39)		
vehicles					areas, lower in support areas
Used pesticide cream/spray on skin	12	42	32	52	Unknown; reasonable that it was reported by few at sea.
Wore uniforms treated with pesticides	3	19	3	23	Unknown; reasonable that it was reported by few at sea.
Wore a flea collar	0	2	0	4	Unknown
Saw living area sprayed with pesticides	3	17	23	22	Unknown; reasonable that it was reported by few at sea.
Received shot(s) in arm while in theater	46	67	68	67	Unknown
Received shot(s) in buttocks while in theater	17	31	27	42	Unknown
Took NAPP pills (Pyridostigmine Bromide)	20	58	19	71	Reasonable: Highest in Iraq/Kuwait, less in E. Saudi. Unknown if those at sea, distant support areas took PB
Used/exposed to CARC paint	7	4	3	35	Unknown
Frequently got < 4 hrs sleep in 4 hrs	38	39	37	73	Reasonable: Highest in battlefield areas, lower in support areas

Table 28. Proportion of Gulf War Veterans in Study 1 Reporting Individual Exposures, by Veterans' Reported Time Period in Theater

	Time Period In Theater					
% of subjects reporting exposure to:	Departed before Jan 1991 (n=4)	Present Jan/Feb, out in Mar 1991 (n=58)	Present Jan/Feb, out in Apr/May 1991 (n=168)	Present Jan/Feb, out in Jun/Jul 1991 (n=51)	Arrived after Feb 1991 (n=9)	Comments
Smoke from oil well fires	0	53	82	78	50	Unknown overall, but reasonable that exposures reported only Jan-Feb and later
Heard chemical alarms	33	45	63	52	0	Reasonable: rare prior to Jan, none after Feb 1991
SCUD exploded within 1 mile	0	29	42	51	0	Unknown overall, but reasonable that none prior to Jan, none after Feb 91
Directly involved in ground combat	0	23	34	24	0	Reasonable: none prior to Jan, 23%-34% of troops in Jan-Feb, none after Feb 91
Directly involved in air combat	0	7	4	10	0	Reasonable: low % overall, none before and after air war
Saw U.S./allied troops killed/wounded	0	30	43	30	22	Unknown overall, similar to proportions in large national study
Saw Iraqis/civilians killed/wounded	0	30	61	61	22	Unknown
Had contact with prisoners of war	0	28	54	55	0	Unknown overall, but reasonable that none prior to Jan, none after Feb 91
Saw or had contact with dead animals	25	26	49	47	22	Unknown
Saw destroyed enemy vehicles	0	37	77	75	33	Unknown
Had contact with destroyed enemy vehicles	0	23	54	59	22	Unknown
Used pesticide cream/spray on skin	50	36	45	53	22	Unknown, but lower than usage reported in RAND study
Wore uniforms treated with pesticides	0	7	23	18	0	Unknown
Wore a flea collar	0	0	3	6	0	Reasonable: all studies indicate this was rare
Saw living area sprayed with pesticides	25	17	21	26	0	Unknown
Received shot(s) in arm while in theater	25	71	63	69	44	Unknown, but may be high. DOD records indicate that considerably lower % received anthrax/botulinum vaccines during deployment. Most other shots given in the arm were administered prior to deployment.
Received shot(s) in buttocks while in theater	0	38	38	30	33	As above.
Took NAPP pills (PB)	50	36	69	47	11	Unknown overall, RAND study indicated that

	Time Period In Theater					
% of subjects reporting exposure to:	Departed before Jan 1991 (n=4)	Present Jan/Feb, out in Mar 1991 (n=58)	Present Jan/Feb, out in Apr/May 1991 (n=168)	Present Jan/Feb, out in Jun/Jul 1991 (n=51)	Arrived after Feb 1991 (n=9)	Comments
						about 50% used PB in theater. Unlikely that it was used prior to Jan 91
Used/exposed to CARC paint	0	6	28	28	0	Unknown
Frequently got < 4 hrs sleep in 4 hrs	25	43	64	58	50	Unknown

Overall, veterans' responses to questions about exposures in theater that can be evaluated on the basis of known facts about the war appeared to be generally reasonable. These types of evaluations cannot address the accuracy of individual reports of exposures. However, they do provide reassurance that, on the whole, veterans in the study did not indiscriminately report multiple exposures that do not coincide in any way with what was expected based on what is known about the Gulf War. This is especially so for the minority of veterans in the study who were not present in theater in January or February of 1991, during the period of active hostilities. Very few of these individuals reported any of the exposures about which they were asked. In addition, responses to several questions indicate that, for exposures about which veterans should have definitively known their exposure status at the time of the war (e.g., participation in ground combat, contact with destroyed enemy vehicles) no or very few individuals responded in a way that was inconsistent with known facts about the war.

It is extremely important to be aware of information biases likely introduced by inaccurate self-reporting of exposures in this and other studies. The magnitude of the effects of these biases on estimates of associations between GWI and exposures in theater cannot be precisely determined. If exposures were both "over reported" and "under-reported" equally by both Cases and Controls (i.e., nondifferential misclassification) findings would likely underestimate the actual strength of association between exposure and GWI (i.e., odds ratios for actual risk factors would appear lower than they actually should be). In contrast, if exposures were "over reported" by Cases and "under-reported" by Controls, results would likely overestimate the actual strength of association between exposure and GWI (i.e., odds ratios for actual risk factors would appear higher than they actually should be).

If veterans' reports of exposures during deployment had appeared to be generally unreasonable, it is doubtful that analyses of relationships between exposures and GWI could provide useful information. However, the overall reasonableness of veterans' reports of deployment-related exposures/experiences in this study provides an indication that analyses relevant to exposures can be useful, keeping in mind the limitations and caveats described.

Multivariable Analyses to Evaluate Associations Between Self-reported Exposures and GWI Case/Control Status. Logistic regression analyses were conducted using Study 1 data to assess independent relationships between self-reported exposures and GWI Case status, controlling for possible confounding effects of multiple correlated exposures. As previously described, the majority of self-reported exposures had appeared to be associated with GWI Case status in bivariate analyses. Consequently, the modeling procedure began with inclusion of all individual exposures as main effects. Individual variables were eliminated from the model sequentially, in reverse order of their magnitude of association with GWI Cases status at each stage. Table 29 below provides results of both the unadjusted (bivariate) and adjusted (multivariable) analyses, with statistically significant associations indicated in bold type.

Table 29. Association of Self-Reported Exposures with GWI Case Status in Study 1: All Gulf War Veterans

	OR (95% C.I.) Unadjusted	OR (95% C.I.) Adjusted*
Smoke from oil well fires	**2.40 (1.41-4.11)**	1.28 (0.65-2.52)
Heard chemical alarms sounded	1.31 (0.83-2.07)	0.61 (0.33-1.11)
SCUD missile exploded within 1 mile	**2.10 (1.30-3.39)**	**1.94 (1.12-3.37)**
Directly involved in ground combat	1.42 (0.86-2.36)	**0.43 (0.21-0.88)**
Directly involved in air combat	1.27 (0.48-3.38)	1.02 (0.31-3.42)
Saw U.S./allied troops badly wounded or killed	1.31 (0.82-2.10)	0.73 (0.39-1.38)
Saw Iraqis/civilians badly wounded or killed	**2.71 (1.70-4.31)**	**2.10 (1.12-3.94)**
Had contact with prisoners of war	**2.62 (1.64-4.17)**	1.68 (0.83-3.41)
Saw or had contact with dead animals	**2.20 (1.38-3.51)**	1.42 (0.78-2.61)
Had direct contact with destroyed enemy vehicles	**2.63 (1.65-4.18)**	1.27 (0.66-2.46)
Used pesticide cream or spray on skin	**2.89 (1.80-4.65)**	1.52 (0.84-2.76)
Wore a uniform treated with pesticides	**3.72 (1.91-7.21)**	**2.75 (1.31-5.78)**
Wore a flea collar	8.12 (0.99-66.77)	4.31 (0.45-41.67)
Saw living area fogged/sprayed with pesticides	1.33 (0.74-2.37)	1.21 (0.60-2.45)
Received one or more shots in arm while in theater	**2.00 (1.21-3.30)**	1.52 (0.84-2.76)
Received one or more shots in buttocks while in theater	**1.82 (1.12-2.98)**	1.23 (0.69-2.22)
Took NAPP pills (PB)	**3.21 (1.97-5.24)**	**3.18 (1.73-5.86)**
Used/came into contact with freshly applied CARC paint	**2.04 (1.14-3.63)**	0.99 (0.46-2.12)
Frequently had less than 4 hours sleep in 24 hour period	**2.23 (1.39-3.59)**	**2.07 (1.14-3.76)**
Regular smoker during deployment	1.46 (0.91-2.39)	1.25 (0.70-2.22)

* ORs adjusted for being within 1 mile of exploding SCUD missile, participating in ground combat, witnessing civilian casualties, wearing uniforms treated with pesticides, using PB, and frequently having less than 4 hours sleep in a 24 hour period

As shown, bivariate analyses indicated that the majority of self-reported exposures appeared to be associated with GWI Case status. However, as has been noted by many epidemiologists, and explored more fully in this data set (see below), it was reasonable to assume that many of the apparent associations might have been the result of confounding between exposure variables that occurred in groups. This assumption was proven valid when the use of a multivariable modeling reduced or eliminated the apparent GWI risk associated with exposure to oil well fire smoke, participation in ground combat, contact with prisoners of war, contact with dead animals, contact with destroyed enemy vehicles, use of pesticides on the skin, receiving shots in theater, and exposure to CARC paint. The final model indicated that the exposure associated with highest GWI risk was use of PB (OR=3.18), followed by wearing a uniform treated with pesticides (OR = 2.75). Additional significant risk factors included being within one mile of an exploding SCUD missile, seeing badly wounded or killed Iraqis or civilians, and frequently having less than four hours sleep in a 24-hour period. An unexpected and puzzling finding relates to GWI risk in association with direct participation in ground combat, which had appeared to be mildly elevated in unadjusted analyses. However, when this variable was combined with others in the model, Gulf veterans who had participated in ground combat were found to have a significantly *lower* GWI risk than veterans who had not. We have no explanation for this finding. Participation in ground combat was reported by about half of the personnel who entered Iraq and/or Kuwait, but not by any individuals who were not in those countries. It is possible that the apparent lower risk associated with being a combatant may be the result of a "healthy warrior"-type effect or to

other factors not included in the model. Including a variable for age in the model, however, did not change observed results. The finding is compatible with those from earlier studies indicating that GWI rates are not significantly associated with participation in combat. Overall, this and other wartime experiences that might be considered major deployment-related sources of stress or trauma (hearing chemical alarms, participation in air combat, witnessing U.S. or allied casualties or deaths) did not contribute substantially to the risk of GWI in this study.

As described above, exposures in theater tended to occur in clusters or groups, primarily associated with where veterans served during the war and their branch of service. In particular, personnel who had served in Iraq and/or Kuwait, countries in which all battles took place, reported experiencing a group of 14 exposures significantly more often than veterans who served in other areas of theater. As previously described, preliminary bivariate analyses also suggested that GWI risk factors may differ in relation to deployment to different areas of theater. To further investigate this possibility, we conducted logistic regression analyses to evaluate independent associations between exposures and GWI in subgroups defined by the locations in which veterans served during the war. To allow maximum power for these analyses, location subgroups were collapsed into two categories: veterans who reported entering Iraq and/or Kuwait (n=177), and veterans who were not in Iraq or Kuwait (n=127).

As shown in Table 30, individual exposures significantly associated with GWI varied substantially in relation to whether veterans had served in battlefield areas or not. Among veterans who had served in Iraq and/or Kuwait, the risk of GWI was significantly associated with four exposures: use of PB (OR=5.42), being within one mile of an exploding SCUD missile (OR=2.68), having contact with prisoners of war (OR=2.60), and receiving one or more shots in the arm while in theater (OR=2.25), results that differed somewhat from those observed in the entire sample of Gulf War veterans.

Very few self-reported exposures were elevated—significantly or otherwise—among the group of veterans who had not served in battlefield areas. However, one self-reported exposure stands out in dramatic contrast. The one significant risk factor for GWI in the group of veterans who had not served in Iraq or Kuwait was wearing uniforms treated with pesticides (OR=12.7). It is known that some Gulf War personnel, almost exclusively those in the Army—wore uniforms treated with pyrethroid compounds during deployment.

Table 30. Association of Self-Reported Exposures with GWI Case Status Among Gulf War Veterans from Study 1 Who Did/Did Not Serve in Battlefield Areas

	All Gulf Veterans (n=304)	**Veterans Located in Iraq and/ or Kuwait** (n=177)	**Veterans Who Did not Enter Iraq or Kuwait** (n=127)
	Adjusted* OR (95% C.I.)	Adjusted** OR (95% C.I.)	Adjusted*** OR (95% C.I.)
Smoke from oil well fires	1.28 (0.65-2.52)	3.04 (0.99 – 9.36)	1.36 (0.60-3.10)
Heard chemical alarms sounded	0.61 (0.33-1.11)	0.62 (0.29 – 1.32)	1.01 (0.44-2.30)
SCUD missile exploded within 1 mile	**1.94 (1.12-3.37)**	**2.68 (1.28 – 5.63)**	1.08 (0.46-2.55)
Directly involved in ground combat	**0.43 (0.21-0.88)**	**0.25 (0.10 – 0.64)**	--

	All Gulf Veterans (n=304)	Veterans Located in Iraq and/ or Kuwait (n=177)	Veterans Who Did not Enter Iraq or Kuwait (n=127)
	Adjusted* OR (95% C.I.)	Adjusted** OR (95% C.I.)	Adjusted*** OR (95% C.I.)
Directly involved in air combat	1.02 (0.31-3.42)	0.75 (0.18 – 3.24)	2.71 (0.37-20.20)
Saw U.S/allied troops badly wounded or killed	0.73 (0.39-1.38)	0.97 (0.46 – 2.07)	0.94 (0.35-2.48)
Saw Iraqis/civilians badly wounded or killed	**2.10 (1.12-3.94)**	1.72 (0.68 – 4.36)	1.00 (0.30-3.31)
Had contact with prisoners of war	1.68 (0.83-3.41)	**2.60 (1.06 – 6.38)**	0.83 (0.24-2.82)
Saw or had contact with dead animals	1.42 (0.78-2.61)	1.41 (0.65 – 3.03)	0.87 (0.30-2.49)
Had direct contact with destroyed enemy vehicles	1.27 (0.66-2.46)	1.43 (0.62 – 3.34)	1.80 (0.44-7.37)
Used pesticide cream or spray on skin	1.52 (0.84-2.76)	1.81 (0.88 – 3.76)	1.62 (0.63-4.17)
Wore a uniform treated with pesticides	**2.75 (1.31-5.78)**	1.65 (0.68 – 3.99)	**12.74 (2.64-61.49)**
Wore a flea collar	4.31 (0.45-41.67)	4.47 (0.44 – 45.91)	--
Saw living area fogged/sprayed with pesticides	1.21 (0.60-2.45)	1.21 (0.49 – 2.98)	0.72 (0.22-2.40)
Received one or more shots in arm while in theater	1.52 (0.84-2.76)	**2.25 (1.04 – 4.85)**	1.52 (0.62-3.72)
Received one or more shots in buttocks while in theater	1.23 (0.69-2.22)	1.12 (0.48 – 2.60)	1.27 (0.50-3.22)
Took NAPP pills (PB)	**3.18 (1.73-5.86)**	**5.42 (2.17 – 13.51)**	1.44 (0.59-3.47)
Used/came into contact with freshly applied CARC paint	0.99 (0.46-2.12)	1.07 (0.49 – 2.35)	1.04 (0.14-7.84)
Frequently had less than 4 hours sleep in 24 hour period	**2.07 (1.14-3.76)**	2.03 (0.86 – 4.79)	1.56 (0.67-3.66)
Regular smoker during deployment	1.25 (0.70-2.22)	0.86 (0.41 – 1.80)	1.49 (0.65-3.40)

*ORs adjusted for being within 1 mile of exploding SCUD missile, participating in ground combat, witnessing civilian casualties, wearing uniforms treated with pesticides, using PB, and frequently having less than 4 hours sleep in a 24 hour period

**ORs adjusted for experiencing oil fire smoke, scud missile within 1 mile, contact with prisoners of war, receiving shot in arm while in theater, taking NAPP pills, and engaging in ground combat

***ORs adjusted for wearing a uniform treated with pesticides

Assessment of Exposure Combinations in Relation to Case/Control Status in Study 1: Pesticides and PB. Gulf War veterans were asked whether they had used PB tablets during deployment, and whether they had been exposed to any of three sources of pesticides: pesticides used on their skin, pesticides sprayed on their uniforms, and having their living area sprayed or fogged with pesticides. Multivariable analyses indicated that PB and wearing uniforms treated with pesticides were the two variables most strongly associated with GWI overall, with PB being associated with the highest risk among those who served in battlefield areas, and uniforms with pesticides being the dominant exposure of concern among those in support areas. Using pesticides creams or sprays on the skin was significantly associated with GWI in unadjusted analyses in the total sample as well as in both veteran subgroups, and somewhat elevated in adjusted analyses. Because risk associated with these exposures appeared to differ somewhat in relation to deployment location, and because multiple animal studies have indicated that these types of exposures may act synergistically when used in conjunction with one another, additional analyses were undertaken to determine whether Case status was differentially associated with combinations of reported use of PB, use of pesticide cream or sprays on the skin (presumably including DEET products), and wearing uniforms treated with pesticides (presumably primarily pyrethroid products). Results are summarized in Table 31.

Table 31. Evaluation of Combined Effects of Two Sources of Pesticides in Theater and PB in Relation to Study 1 GWI Case Status

	n	% Cases Exposed (n = 144)	% Controls Exposed (n=160)	OR (95% CI)
No skin pesticides, no uniform pesticides, no PB	82	15	42	1.0
Skin pesticides only, no uniform, no PB	27	8	11	1.82 (0.72-4.62)
Uniform pesticides only, no skin, no PB	2	0	1	--
PB only, no skin or uniform pesticides	75	28	26	**3.02 (1.53-5.94)**
Skin pesticides and PB, no uniform pesticides	48	22	13	**4.73 (2.20-10.19)**
Uniform pesticides and PB, no skin pesticides	3	2	1	--
Skin and uniform pesticides, no PB	9	5	1	**10.85 (2.08-56.51)**
Skin and uniform pesticides, with PB	35	20	5	**10.46 (4.10-26.68)**

Evaluation of the results should be interpreted with caution, in light of the sample size and number of exposure subgroups evaluated. The analyses presented suggest that there is an interactive effect between the use of pesticides on the skin and wearing pesticide-treated uniforms. That is, the relatively mild risk of GWI associated with reports of using pesticides on the skin during deployment was significantly enhanced among personnel who also reported wearing uniforms treated with pesticides. This interactive effect was not further enhanced by reported use of PB in this sample.

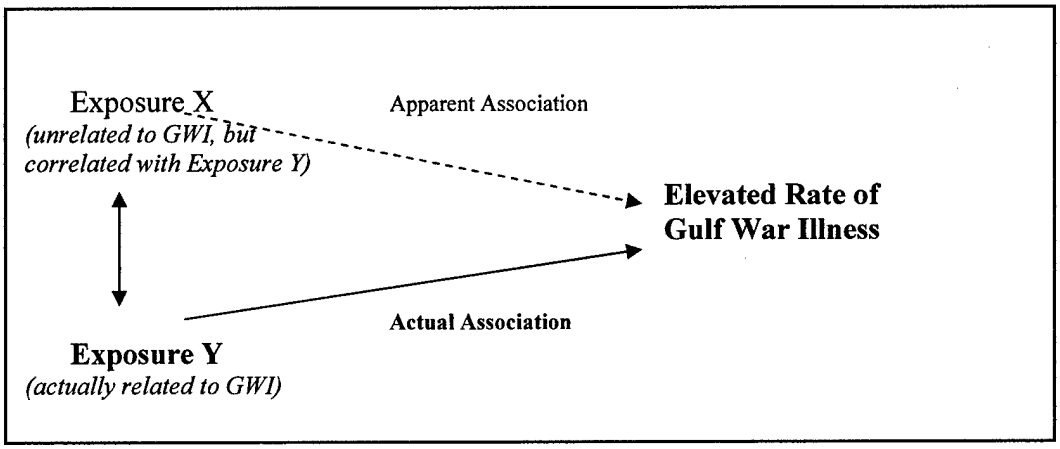
Veterans in this study rarely reported wearing uniforms treated with pesticides without also having used pesticide creams/sprays on their skin. Therefore, it was not possible to identify the level of risk that may have been associated with pesticide-treated uniforms in the absence of skin pesticides (with or without using PB). The use of skin pesticides alone may be associated with a mildly elevated risk for GWI (nonsignificant OR of 1.82). GWI risk was substantially higher among veterans who reported both that they used pesticides on their skin and wore pesticide-treated uniforms during deployment (OR = 10.85). However, since it was not possible to identify the level of effect associated with pesticide-treated uniforms alone, the possibility that the observed elevated risk may be entirely due to the effects of the treated uniforms (independent of possible interaction with skin pesticides) cannot be ruled out. The analyses summarized above also suggest that skin pesticides and PB together may have an additive effect, with the risk of both together being similar to the sum of the two exposures individually.

Multivariable analyses indicated that only a limited number of self-reported exposures were significantly associated with elevated risk of GWI in this study. Overall, the greatest risk factor for GWI was use of PB during deployment, followed by wearing uniforms treated with pesticides. Logistic regression analyses in mutually exclusive groups of veterans deployed to different areas of theater indicated that the relative importance of different GWI risk factors may vary in different veteran subgroups. Among veterans who served in battlefield areas, GWI was most strongly associated with use of PB, proximity to exploding SCUD missiles, contact with prisoners of war, and receipt of shots in the arm during deployment. In contrast, wearing uniforms treated with pesticides was most strongly linked to elevated risk of GWI among veterans who served exclusively in support areas. In addition, analyses

indicated that veterans who reported both the use of pesticides applied to the skin and wearing uniforms treated with pesticides had a substantially greater risk for GWI than veterans who reported use of skin pesticides alone. Taken together, our findings suggest that relationships between exposures and GWI are complex. However, careful use of modeling, subgroup analyses, and assessment of the effects of combined exposures have the potential to shed considerable light on these relationships.

Evaluation of the Potential Role of Exposure Subgroups and Confounding in Identifying Associations of GWI with Exposures and Characteristics of Military Service. Epidemiologic studies have often reported that veterans' self-reported exposures during Gulf War deployment are linked to excess rates of illness and symptoms. A number of large epidemiologic studies have reported results of bivariate analyses assessing relationships between self-reported exposures and multisymptom illness. These analyses typically indicate that a large variety of self-reported exposures appear to be associated with GWI[59,60]. However, several studies and reports[61,62] have indicated that Gulf War-related exposures tend to be highly correlated with one another. That is, groups of veterans who experienced some exposures during the war were also more likely to have experienced other specific types of exposures. If exposures in theater were highly correlated, the expected effect would be that some apparent associations between illness and self-reported exposures identified in bivariate analyses could be due to confounding, that is, errors introduced when an apparent association between two variables is actually due to an additional factor not accounted for by the analysis, as shown in Figure 13 below. Identifying patterns of concurrent exposures, then, is essential to teasing out complex relationships that may exist between GWI and factors related to deployment.

Figure 13. Confounding Can Lead to Errors in Interpreting Apparent Associations Between GWI and Self-Reported Exposures in Epidemiologic Studies



This possibility has been borne out by reports that have used more sophisticated multivariable analyses to identify exposures that are independently associated with GWI, while controlling for effects of confounding. Such studies typically find a much smaller number of self-reported exposures to be associated with illness in Gulf War veterans.

We set out to evaluate the extent to which correlations between variables might be responsible for confounding in apparent associations between self-reported exposures and Gulf War veterans' illnesses. We further evaluated whether exposure groupings might be important in relation to GWI, and

which individual exposures within those groupings appeared to be independently associated with excess illness in GWI Cases vs. DC. NDC were not considered in these analyses for obvious reasons, since they were, by definition, not ill and had not experienced the majority of Gulf War-related exposures. These analyses also focused on data collected for Study 1, since it included the largest number of veterans who had been randomly selected from among all those who deployed to the Gulf War.

Associations Between GWI and Characteristics of Military Service, Self-Reported Exposures - Bivariate Analyses. As shown in the tables below, bivariate analyses of Study 1 data suggested that there are a number of significant associations between GWI and characteristics of military service (branch of service, rank) and the locations in which veterans served during the war. GWI appeared to be linked most prominently to being in the Army, being in the enlisted ranks and having spent some period of time in Iraq and/or Kuwait during deployment. Serving with the Navy during the war, and being located at sea at least one week during deployment was associated with a significantly lower rate of GWI. In addition, bivariate analyses indicate that GWI was significantly associated with 15 of the 18 individual exposures about which veterans were asked. Exposure to various forms of pesticides and use of PB pills were most highly associated with GWI in these analyses.

Table 32. Association of Case Status with Military and Deployment Characteristics in Study 1

	All PGW Veterans (144 Cases, 160 Controls)		
	% Cases	% Controls	OR (95% C.I.)
Military Characteristics			
Army	65%	47%	**2.07 (1.30 – 3.28)**
Navy	10%	21%	**0.43 (0.22 – 0.83)**
Air Force	8%	11%	0.69 (0.31 – 1.54)
Marines	17%	21%	0.81 (0.45 – 1.44)
Enlisted rank (vs. officers)	86%	73%	2.28 (1.27 – 4.10)
Characteristics of Deployment			
At sea (one week or more)	9%	18%	0.44 (0.21 – 0.90)
In Kuwait	64%	39%	2.80 (1.75 – 4.49)
In Iraq	46%	32%	1.80 (1.35 – 4.96)
In Eastern Saudi Arabia	89%	76%	2.59 (1.35 – 4.96)
In Central Saudi Arabia	55%	44%	1.60 (1.01 – 2.54)
In Bahrain	57%	55%	1.09 (0.69 – 1.73)
In Northern Saudi Arabia	22%	23%	0.94 (0.54 – 1.64)
In Western Saudi Arabia	10%	12%	0.81 (0.39 – 1.68)
In Iraq or Kuwait	70%	47%	2.60 (1.62 – 4.17)
Mutually Exclusive Location Categories:			
Not in Iraq or Kuwait:			
At sea ≥ 1 week	4%	16%	1.0
In Eastern Saudi ≥ 1 wk	15%	21%	2.80 (0.99 – 7.91)
On land, other	10%	15%	2.70 (0.90 – 8.11)
Entered Iraq or Kuwait	70%	48%	5.76 (2.26–14.69)

Table 33. Bivariate (unadjusted) Associations between GWI and Exposures Reported by Gulf War Veterans in Study 1

Exposure	% Cases Exposed	% Controls Exposed	OR (95% C.I.)
Wore a flea collar	5%	1%	**8.13 (1.00 – 66.93)**
Wore a uniform treated with pesticides	27%	9%	**3.72 (1.91 – 7.21)**
Took NAPP (PB) pills	72%	44%	**3.21 (1.97 – 5.24)**
Used pesticide cream/spray on skin	57%	31%	**2.89 (1.80 – 4.64)**
Saw Iraqis or civilians badly wounded or killed	65%	40%	**2.71 (1.70 – 4.31)**
Had direct contact with destroyed enemy vehicles	60%	36%	**2.63 (1.65 – 4.18)**
Had contact with prisoners of war	59%	35%	**2.62 (1.64 – 4.17)**
Exposed to smoke from oil well fires	82%	65%	**2.40 (1.41 – 4.11)**
Frequently had less than 4 hrs sleep in 24-hrs	69%	49%	**2.24 (1.39 – 3.59)**
Saw/had contact with dead animals	54%	34%	**2.20 (1.38 – 3.51)**
Saw destroyed enemy vehicles	74%	58%	**2.11 (1.29 – 3.43)**
SCUD missile exploded within 1 mile	48%	31%	**2.10 (1.30 – 3.39)**
Used/had contact with fresh CARC (chemical agent resistance coating) paint	29%	17%	**2.04 (1.14 – 3.63)**
Received one or more shots in the arm in theater	73%	58%	**2.00 (1.21 – 3.29)**
Received one or more shots in buttocks in theater	43%	29%	**1.82 (1.12 – 2.98)**
Directly involved in ground combat	32%	25%	1.42 (0.86 – 2.36)
Saw living area sprayed/fogged with pesticides	22%	17%	1.33 (0.74 – 2.37)
Saw U.S. or allied troops badly wounded or killed	39%	33%	1.31 (0.82 – 2.11)
Heard chemical alarms sounded	59%	53%	1.31 (0.83 – 2.07)
Directly involved in air combat	6%	5%	1.27 (0.48 – 3.38)

Correlations Between Exposure Variables, Subgroups of Interest. The first step in identifying potential exposure groupings of interest was to run correlation matrices to evaluate the extent to which different exposures were correlated with one other, and with veterans' military characteristics (branch of service, rank) and locations in theater. Overall, an extensive number of significant correlations were identified. For example, self-reported exposure to smoke from oil well fires was positively and significantly correlated with 13 of the other 18 exposures queried, as well as with veterans being located in Iraq/Kuwait and with being in the Army, and significantly negatively correlated with other locations in theater and being in the Navy or Air Force. Similarly, hearing chemical alarms during deployment was significantly associated with 12 of the 18 exposures queried, and with being located in Iraq/Kuwait, or Eastern Saudi Arabia and with serving in the Army. Taking PB tablets was positively and significantly associated with 14 of the 18 exposures queried, with being located in Iraq/Kuwait, and with being in the Army or Marines. These findings indicated that not only were exposures frequently correlated with many other exposures, they were also significantly associated with location in theater and branch of service.

Chi square tests were then done to determine the extent to which apparent differences in self-reported exposures by branch of service and by location in theater were statistically significant. These results are summarized in Table 34 below. As shown, veterans' reports of entering Iraq and/or Kuwait

during the war, and with serving in the Army were each significantly associated with 14 of 18 exposures queried.

Table 34. Association of Self-Reported Exposures with Gulf War Veterans' Location in Theater and Branch of Service in Study 1

	Location in Theater				Branch of Service			
	Not in Iraq or Kuwait							
Exposures:	At sea ≥ 1 wk (n=32)	E. Saudi ≥ 1 wk (n=56)	On land, other (n=39)	In Iraq or Kuwait (n=177)	Army (n=168)	Navy (n=49)	Air Force (n=28)	Marines (n=58)
Smoke from oil well fires	-- --		-- --	++	+		-- --	
Heard chemical alarms	-- --	++	-- --		++	--	--	
SCUD exploded within 1 mile	-- --	+			+			
Directly involved in ground combat	-- --	-- --	-- --	++	+	-- --	-- --	++
Directly involved in air combat		--					+	
Saw U.S./allied troops killed/wounded	-- --			++	+		-- --	
Saw Iraqis/civilians killed/wounded	-- --	-- --	-- --	++	++	-- --	-- --	
Had contact with prisoners of war	-- --	--	-- --	++	++	-- --	-- --	
Saw or had contact with dead animals	-- --	--	-- --	++	++	-- --	-- --	
Saw destroyed enemy vehicles	-- --	-- --	-- --	++	++	-- --	-- --	
Had contact with destroyed enemy vehicles	-- --	-- --	-- --	++	++	-- --	-- --	
Used pesticide cream/spray on skin	-- --			++	++	-- --		--
Wore uniforms treated with pesticides	--		--	++	++	--	--	
Wore a flea collar								
Saw living area sprayed with pesticides	--						++	
Received shot(s) in arm while in theater	--							
Received shot(s) in buttocks while in theater	--			+				
Took NAPP pills (PB)	-- --		-- --	++	++	-- --	-- --	+
Used/exposed to CARC paint	--	-- --	-- --	++	++	--	-- --	
Frequently got < 4 hrs sleep in 24 hrs	--	-- --	-- --	++			-- --	

\+ = Exposure reported by significantly more individuals in identified group, $p < .05$
++ = Exposure reported by significantly more individuals in identified group, $p < .005$
-- = Exposure reported by significantly fewer individuals in identified group, $p < .05$
-- -- = Exposure reported by significantly fewer individuals in identified group, $p < .005$

We also conducted exploratory factor analyses (orthogonal, varimax rotation) to determine whether latent constructs representing highly correlated groups of exposure variables might be identified. As shown in Table 35 below, the results of the factor analyses generally paralleled those in the chi square analyses. Only the first two factors shown in the table comprised more than two exposure variables. The exposures in those factors generally reflect those previously identified as being highly associated with deployment to Iraq/Kuwait and with serving in the Army. Factor 1 explained the

greatest amount of variance and included most of the exposures associated with serving in Iraq and/or Kuwait. Additional factors reflected constructs that represented correlations between using pesticide cream or spray on the skin and wearing uniforms treated with pesticides, receiving shots in the arm and shots in the buttocks during deployment, and hearing chemical alarms and having a SCUD missile explode within one mile.

Table 35. Study 1: Factor Loadings of Gulf War-related Exposures in Study 1 (values ≥ 0.40 shown)

Exposure:	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Smoke from oil well fires	0.56				
Heard chemical alarms				0.73	
SCUD exploded w/in 1 mile				0.75	
Directly involved in ground combat	0.70				
Directly involved in air combat					0.45
Saw U.S./allied troops killed/wounded	0.45				
Saw Iraqis/civilians killed/wounded	0.75				
Had contact with prisoners of war	0.69				
Saw or had contact with dead animals	0.55				
Saw destroyed enemy vehicles	0.76				
Had contact with destroyed enemy vehicles	0.74				
Used pesticide cream/spray on skin		0.66			
Wore uniforms treated with pesticides		0.76			
Wore a flea collar					
Saw living area sprayed with pesticides					0.68
Received shot(s) in arm while in theater			0.78		
Received shot(s) in buttocks while in theater			0.82		
Took NAPP pills (PB)					
Used/exposed to CARC paint		0.66			
Frequently got < 4 hrs sleep in 24 hrs	0.63				

These analyses, taken together, verify earlier indications that a substantial potential for errors introduced by confounding and colinearity exists in overly-simplified assessments of associations between GWI and multiple exposures encountered during the Gulf War. Further, it raises the possibility that the higher prevalence of GWI observed in previous studies among veterans who served in the Army and who served in Iraq and Kuwait may be the result of individual or groups of exposures more commonly encountered in Iraq/Kuwait, or by Army personnel.

Association of GWI with Exposures and Deployment Characteristics in Subgroups of Interest. Bivariate analyses indicate that GWI is significantly associated both with self-reported exposures and with branch of service and deployment location. A first step often used in evaluating the potential for confounding or interaction/effect modification to underlie apparent associations between

exposures and health status is to assess those associations in subgroups of interest. The sample size of the present study does not adequately support multivariable modeling to determine independent associations of all exposure variables of interest with GWI in all subgroups of interest. In addition, the multiple comparisons involved in such analyses might introduce spurious associations on the basis of chance alone. Therefore, evaluation of the overall potential for GWI risk factors to differ in different subgroups of interest was *conducted as a hypothesis-generating exercise*, using bivariate (unadjusted) analyses and 95% confidence intervals, to provide a preliminary indication of whether exposure/GWI associations may differ in different veteran subgroups. Results of these *hypothesis-generating* evaluations are summarized in Tables 36 and 37.

Table 36. Association of GWI Case Status with Exposures and Military Characteristics, By Branch of Service, in Study 1. Bivariate (unadjusted) Odds Ratios and Confidence Intervals

	Overall	**Army (n=168)**	**Navy (n=49)**	**Air Force (n=28)**	**Marines (n=58)**
	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)
Exposures					
Smoke from oil well fires	2.40 (1.41 – 4.11)	2.16 (1.03 – 4.54)	4.79 (0.93 – 24.71)	1.11 (0.22 – 5.62)	2.75 (0.66 – 11.48)
Heard chemical alarms sounded	1.31 (0.83 – 2.07)	1.48 (0.78 – 2.81)	1.60 (0.47 – 5.51)	0.10 (0.01 – 1.00)	0.87 (0.30 – 2.51)
SCUD missile exploded within 1 mile*	2.10 (1.30 – 3.39)	2.65 (1.39 – 5.04)	7.43 (1.91 – 28.94)	0.49 (0.08 – 3.15)	0.37 (0.10 – 1.38)
Directly involved in ground combat*	1.42 (0.86 – 2.36)	0.78 (0.41 – 1.50)	--	--	2.23 (0.77 – 6.44)
Directly involved in air combat	1.27 (0.48 – 3.38)	0.81 (0.16 – 4.13)	1.14 (0.10 – 13.67)	5.25 (0.46 – 59.29)	1.41 (0.18 – 10.78)
Saw U.S/allied troops badly wounded or killed	1.31 0.82 – 2.11)	0.88 (0.47 – 1.64)	2.84 (0.78 – 10.30)	1.50 (0.08 – 26.86)	1.57 (0.54 – 4.59)
Saw Iraqis/civilians badly wounded or killed	2.71 (1.70 – 4.31)	1.61 (0.81 – 3.21)	3.76 (0.72 – 19.51)	0.70 (0.06 – 8.82)	5.55 (1.77 – 17.38)
Had contact with prisoners of war	2.62 (1.64 – 4.17)	1.90 (1.01 – 3.56)	1.45 (0.30 – 7.05)	--	4.25 (1.40 – 12.88)
Saw or had contact with dead animals	2.20 (1.38 – 3.51)	1.64 (0.88 – 3.04)	2.80 (0.67 – 11.75)	--	2.14 (0.73 – 6.32)
Saw destroyed enemy vehicles*	2.11 (1.29 – 3.43)	1.03 (0.47 – 2.25)	4.41 (1.19 – 16.36)	0.49 (0.08 – 3.15)	4.37 (1.23 – 15.58)
Had direct contact with destroyed enemy vehicles	2.63 (1.65 – 4.18)	1.97 (1.04 – 3.73)	8.00 (1.34 – 47.77)	1.56 (0.18 – 13.11)	2.23 (0.77 – 6.44)
Used pesticide cream or spray on skin	2.89 (1.80 – 4.64)	2.68 (1.42 – 5.06)	4.83 (1.11 – 21.01)	0.73 (0.15 – 3.55)	2.65 (0.83 – 8.50)
Wore a uniform treated with pesticides*	3.72 (1.91 – 7.21)	2.27 (1.08 – 4.77)	--	--	--
Wore a flea collar	8.13 (1.00 – 66.93)	5.16 (0.61 – 43.87)	--	--	--
Saw living area fogged/sprayed with pesticides	1.33 (0.74 – 2.37)	1.37 (0.60 – 3.15)	3.87 (0.73 – 20.35)	0.86 (0.17 – 4.27)	1.35 (0.34 – 5.30)
Received one or more shots in arm while in theater*	2.00 (1.21 – 3.29)	2.18 (1.13 – 4.20)	13.93 (1.61–120.83)	0.22 (0.04 – 1.21)	1.78 (0.52 – 6.13)

	Overall	Army (n=168)	Navy (n=49)	Air Force (n=28)	Marines (n=58)
	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)
Exposures					
Received one or more shots in buttocks while in theater*	1.82 (1.12 – 2.98)	1.18 (0.63 – 2.21)	8.40 (1.72 – 40.91)	0.62 (0.12 – 3.32)	3.65 (1.06 – 12.56)
Took NAPP pills (PB)	3.21 (1.97 – 5.24)	3.27 (1.62 – 6.59)	3.33 (0.57 – 19.47)	0.67 (0.10 – 4.48)	3.16 (0.87 – 11.52)
Used/came into contact with freshly applied CARC paint	2.04 (1.14 – 3.63)	1.74 (0.87 – 3.49)	3.11 (0.38 – 25.38)	--	1.19 (0.27 – 5.35)
Frequently had less than 4 hours sleep in 24 hour period	2.24 (1.39 – 3.59)	1.97 (1.04 – 3.73)	6.46 (1.53 – 27.32)	1.03 (0.19 – 5.68)	1.79 (0.58 – 5.48)
Regular smoker during deployment	1.47 (0.91 – 2.38)	1.26 (0.67 – 2.39)	3.86 (1.01 – 14.69)	0.73 (0.11 – 4.82)	1.20 (0.40 – 3.60)
Military Characteristics					
At sea ≥ 1 week	0.22 (0.09 – 0.56)	--	0.20 0.05 – 0.83)	--	0.61 (0.14 – 2.74)
In Eastern Saudi Arabia ≥ 1 wk*	0.67 (0.37 – 1.21)	1.35 (0.61 – 2.99)	--	0.47 (0.04 – 5.17)	0.13 (0.03 – 0.67)
On land, other*	0.66 (0.33 – 1.31)	0.11 (0.01 – 0.88)	3.11 (0.80 – 12.10)	1.87 (0.36 – 9.63)	--
Entered Iraq or Kuwait	2.59 (1.62 – 4.17)	1.33 (0.65 – 2.70)	5.00 (1.15 – 21.71)	0.72 (0.11 – 4.82)	6.15 (1.85 – 20.50)
Enlisted rank (vs. officer)	2.28 (1.27 – 4.10)	2.37 (1.17 – 4.82)	3.00 (0.33 – 27.40)	1.87 (0.29 – 11.97)	--

-- OR undefined due to 0 cell values
* Association varies significantly by branch of service

Table 37. Association of Case Status With Exposures and Military Characteristics in Study 1 By Location in Theater. Mutually Exclusive Location Subgroups; Bivariate (unadjusted) Odds Ratios and Confidence Intervals

	Overall	At Sea ≥ 1 wk (n = 32)	E. Saudi ≥ 1 wk (n = 56)	On Land, Other (n = 39)	Entered Iraq or Kuwait (n=177)
	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)
Exposures					
Smoke from oil well fires	2.40 (1.41 – 4.11)	1.36 (0.23 – 8.08)	1.85 (0.55 – 6.28)	1.19 (0.29 – 4.90)	2.11 (0.85 – 5.232)
Heard chemical alarms sounded*	1.31 (0.83 – 2.07)	1.81 (0.25 – 13.21)	5.71 (1.13 – 28.80)	0.09 (0.01 – 0.77)	1.10 (0.60 – 2.03)
SCUD missile exploded within 1 mile	2.10 (1.30 – 3.39)	2.30 (0.17 – 30.59)	1.20 (0.40 – 3.56)	0.64 (0.15 – 2.68)	2.74 (1.44 – 5.23)
Directly involved in ground combat	1.42 (0.86 – 2.36)	--	--	--	0.79 (0.44 – 1.45)
Directly involved in air combat	1.27 (0.48 – 3.38)	--	--	3.23 (0.27 – 39.28)	0.88 (0.28 – 2.73)
Saw U.S/allied troops badly wounded or killed	1.31 (0.82 – 2.11)	1.53 (0.13 – 17.97)	0.90 (0.27 – 2.97)	0.71 (0.15 – 3.41)	1.08 (0.59 – 1.98)
Saw Iraqis/civilians badly wounded or killed	2.71 (1.70 – 4.31)	--	2.18 (0.62 – 7.66)	0.75 (0.06 – 9.08)	2.16 (1.04 – 4.48)
Had contact with prisoners of war	2.62 (1.64 – 4.17)	5.00 (0.27 – 93.96)	2.25 (0.70 – 7.19)	--	2.07 (1.09 – 3.92)
Saw or had contact with dead animals	2.20 (1.38 – 3.51)	--	1.39 (0.42 – 4.54)	1.58 (0.27 – 9.17)	1.82 (0.98 – 3.37)
Saw destroyed enemy vehicles	2.11 (1.29 – 3.43)	--	0.61 (0.18 – 2..10)	0.73 (0.12 – 4.59)	5.55 (0.61 – 50.75)
Had direct contact with destroyed enemy vehicles	2.63 (1.65 – 4.18)	--	2.21 (0.52 – 9.34)	--	1.68 (0.85 – 3.35)
Used pesticide cream or spray on skin*	2.89 (1.80 – 4.64)	--	9.90 (2.82 – 34.77)	0.68 (0.16 – 2.85)	2.37 (1.28 – 4.38)
Wore a uniform treated with pesticides*	3.72 (1.91 – 7.21)	--	26.18 (2.97 – 230.82)	--	2.01 (0.94 – 4.28)
Wore a flea collar	8.13 (1.00 – 66.93)	--	--	--	4.79 (0.56 – 40.63)
Saw living area fogged/sprayed with pesticides*	1.33 (0.74 – 2.37)	--	0.16 (0.02 – 1.35)	0.48 (0.08 – 2.86)	1.75 (0.83 – 3.69)
Received one or more shots in arm while in theater	2.00 (1.21 – 3.29)	2.00 (0.27 – 14.59)	3.50 (0.96 – 12.70)	0.79 (0.19 – 3.22)	1.90 (1.00 – 3.63)
Received one or more shots in buttocks while in theater	1.82 (1.12 – 2.98)	4.67 (0.54 – 40.46)	1.46 (0.46 – 4.66)	1.13 (0.26 – 5.01)	1.56 (0.83 – 2.92)
Took NAPP pills (PB)	3.21 (1.97 – 5.24)	11.00 (1.27 – 95.18)	1.87 (0.59 – 5.91)	0.60 (0.10 – 3.61)	3.19 (1.61 – 6.33)
Used/came into contact with freshly applied CARC paint	2.04 (1.14 – 3.63)	5.25 (0.27 – 102.42)	1.61 (0.09 – 27.40)	--	1.50 (0.78 – 2.91)
Frequently had less than 4 hours sleep in 24 hour period	2.24 (1.39 – 3.59)	1.89 (0.31 – 11.34)	2.09 (0.67 – 6.49)	0.93 (0.23 – 3.65)	1.84 (0.94 – 3.60)
Regular smoker during deployment	1.47 (0.91 – 2.38)	2.80 (0.36 – 21.48)	1.12 (0.35 – 3.58)	1.52 (0.33 – 6.96)	1.24 (0.67 – 2.29)

	Overall	At Sea ≥ 1 wk (n = 32)	E. Saudi ≥ 1 wk (n = 56)	On Land, Other (n = 39)	Entered Iraq or Kuwait (n=177)
	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)	OR (95% C.I.)
Military Characteristics					
Army*	2.07 (1.30 – 3.28)	--	11.61 (2.85 – 47.38)	0.18 (0.02 – 1.58)	1.00 (0.51 – 1.94)
Navy	0.43 (0.22 – 0.83)	0.37 (0.06 – 2.27)	--	2.00 (0.50 – 7.80)	1.14 (0.31 – 4.18)
Air Force	0.69 (0.31 – 1.54)	--	0.49 (0.05 – 5.06)	1.60 (0.44 – 5.87)	0.36 (0.06 – 2.04)
Marines*	0.81 (0.45 – 1.44)	3.33 (0.53 – 21.03)	0.16 (0.03 – 0.81)	--	1.20 (0.55 – 2.59)
Enlisted rank (vs. officer)	2.28 (1.27 – 4.10)	--	1.72 (0.30 – 9.78)	8.40 (0.94 – 75.10)	2.72 (1.35 – 5.49)

-- OR undefined, due to zero cell values
* Association differs significantly by location in theater

Results of the subgroup analyses provide a preliminary indication that, overall, excess risk associated with some exposures may vary both by branch of service and by location in theater during deployment. Apparent differences in risk factors for GWI by branch and location provide a number of intriguing possibilities and provide testable hypotheses that can be explored in larger samples.

Preliminary general observations can also be made concerning GWI risk factors in different branches of service. Overall, GWI risk factors for Army veterans are similar to those observed for Gulf War veterans as a whole, with highest illness rates observed in veterans who report use of PB and all forms of pesticides during deployment. Interestingly, direct participation in ground combat was not a risk factor for Army veterans (OR = 0.8). As previously indicated, relatively few Air Force veterans were included in the sample, and these individuals tended to report many of the exposures queried less frequently than veterans from other branches. Among Air Force veterans, only participation in air combat appeared to be potentially related to GWI (OR = 5.25), but this finding was not statistically significant in this small sample.

In contrast, illness risk factors for both Marines and Navy personnel, for whom types and areas of service might have been most diverse (including service in combat areas, land support areas, and service on board ship) were highly variable. Marines at highest risk for GWI appeared to be those who experienced exposures related to combat areas: witnessing enemy casualties (OR=5.6) and destroyed enemy vehicles (OR=4.4), contact with POWs (OR =4.3). Navy personnel at highest risk for GWI were those who reported being in the vicinity of a SCUD missile when it exploded (OR=7.43), and a variety of other distinct exposures such as contact with enemy vehicles (OR=8.0), receipt of shots in the arm (OR=13.9) or buttocks (OR=8.4) during deployment, and frequently having less than 4 hours sleep during deployment (OR=6.5). Despite the relatively small number of Marines and Navy personnel in the sample, these associations were statistically significant.

There are differences in GWI risk factors by location in theater. The greatest risk for GWI among veterans who reported serving in Iraq and/or Kuwait during deployment was associated with use of PB pills, being in the vicinity of a SCUD missile when it exploded, and use of different forms of pesticides. Direct participation in ground combat was not a risk factor for veterans who reported being in battlefield areas. The greatest risk among veterans who served in Eastern Saudi Arabia but did not enter Iraq or Kuwait was found among veterans who reported wearing uniforms treated with pesticides, with an OR of 26.2 - an OR that was both strikingly high and statistically significant. Use of pesticides on the skin was also a significant risk factor for GWI among veterans located in Eastern Saudi Arabia (OR = 9.9).

As previously indicated, many of the exposures about which veterans were asked were infrequently reported by the small sample of veterans who served on board ship at least one week during deployment. The only significant risk factor for GWI identified in this group was use of PB (OR = 11.0). No significant risk factors for GWI were identified among the small number of veterans who served on land in support areas other than Eastern Saudi Arabia.

There were interactions between Branch of Service and location of deployment in association with risk for GWI. Subgroup analyses summarized in Tables 36 and 37 above provide a strong indication of interaction between location in theater and branch of service in association with risk for GWI. Specifically, Army veterans have been shown in this and other studies to be at higher risk for GWI than veterans in other branches. Certainly, previous analyses show that a higher proportion of Army veterans than those in other branches served in areas (Iraq and Kuwait) associated with highest GWI rates. However, the excess risk for GWI identified with battlefield areas does not appear to be associated with being in the Army. That is, Navy, Marines, and Army veterans who report entering Iraq and/or Kuwait during the war all were found to have a similar risk of GWI. However, a strikingly elevated risk for Army veterans relative to those in other branches was found among those who served in Eastern Saudi Arabia but did not enter Iraq or Kuwait, where Army service was associated with a statistically significant OR of 11.6. In contrast, point estimates for ORs among those in other branches who served in Eastern Saudi Arabia without entering Iraq/Kuwait were all below 1.0.

Gulf War-Related Exposures as Risk Factors for GWI: Comparison of Findings from Study 1 and Study 2. This contract undertook two distinct studies (Study 1 and Study 2) of different aspects of GWI in different populations. Study 1 was a random sample of 304 veterans from Kansas and Missouri who had deployed to the Gulf War from different branches of service, including both officers and enlisted personnel who had served throughout the Persian Gulf theater of operations. The Gulf War-deployed participants in the Case/Control portion of Study 2 were randomly sampled Army veterans who had served in the enlisted ranks in one of two units, predominantly including veterans from an infantry division who had all been in battlefield areas. Based on sampling alone, Study 1 would be expected to provide information applicable to the general cohort of veterans who served in the Gulf War, while Study 2 would be expected to provide information more pertinent to those at highest risk for GWI, that is, enlisted Army personnel who had served at or near the battlefront.

As summary results indicate in Table 38, initial bivariate analyses investigating associations between GWI and self-reported exposures *appeared* to provide very different conclusions in Study 2 than in Study 1. When data for exposure variables from Study 2 were collapsed into dichotomous (yes/no) responses, only one wartime exposure—smoking during deployment—was identified as a

significant GWI risk factor. In contrast, bivariate analyses of dichotomous responses using Study 1 data suggested that the majority of exposures about which veterans were asked were significantly associated with GWI. Note that the Controls used in this analysis for Study 2 are the DC.

Table 38. Studies 1 and 2: Association of Exposures in Theater with GWI Case Status

	Study 1 (144 Cases, 160 Controls)			Study 2 (49 Cases, 19 Controls)		
Exposure	**% Cases Exposed**	**% Controls Exposed**	**OR (95% C.I.)**	**% Cases Exposed**	**% Controls Exposed**	**OR (95% C.I.)**
Wore a flea collar	5%	1%	8.13 (1.00 – 66.93)	4%	11%	0.34 (0.04 – 2.62)
Wore a uniform treated with pesticides	27%	9%	3.72 (1.91 – 7.21)	38%	17%	3.10 (0.79–12.23)
Took NAPP (PB) pills	72%	44%	3.21 (1.97 – 5.24)	87%	83%	1.40 (0.31 – 6.31)
Used pesticide cream/spray on skin	57%	31%	2.89 (1.80 – 4.64)	67%	67%	1.03 (0.33 – 3.25)
Saw Iraqis or civilians badly wounded or killed	65%	40%	2.71 (1.70 – 4.31)	86%	89%	0.75 (0.14 – 4.00)
Had direct contact with destroyed enemy vehicles	60%	36%	2.63 (1.65 – 4.18)	82%	72%	1.71 (0.49 – 6.02)
Had contact with prisoners of war	59%	35%	2.62 (1.64 – 4.17)	76%	89%	0.39 (0.08 – 1.92)
Exposed to smoke from oil well fires	82%	65%	2.40 (1.41 – 4.11)	100%	100%	-
Frequently had less than 4 hrs sleep in 24-hrs	69%	49%	2.24 (1.39 – 3.59)	90%	83%	1.76 (0.37 – 8.26)
Saw/had contact with dead animals	54%	34%	2.20 (1.38 – 3.51)	60%	72%	0.59 (0.18 – 1.92)
Saw destroyed enemy vehicles	74%	58%	2.11 (1.29 – 3.43)	96%	100%	-
SCUD missile exploded w/in 1 mile	48%	31%	2.10 (1.30 – 3.39)	40%	28%	1.76 (0.54 – 5.77)
Used/contact w fresh CARC paint	29%	17%	2.04 (1.14 – 3.63)	55%	50%	1.20 (0.40 – 3.60)
Received one or more shots in the arm in theater	73%	58%	2.00 (1.21 – 3.29)	55%	50%	1.23 (0.40 – 3.80)
Received one or more shots in buttocks in theater	43%	29%	1.82 (1.12 – 2.98)	22%	6%	4.44 (0.52–37.71)
Regular smoker during deployment	38%	29%	1.47 (0.91 – 2.38)	63%	21%	6.46 (1.86–22.46)
Directly involved in ground combat	32%	25%	1.42 (0.86 – 2.36)	51%	44%	1.30 (0.44 – 3.85)
Saw living area sprayed/fogged with pesticides	22%	17%	1.33 (0.74 – 2.37)	13%	17%	0.77 (0.17 – 3.48)

	Study 1 (144 Cases, 160 Controls)			Study 2 (49 Cases, 19 Controls)		
Exposure	**% Cases Exposed**	**% Controls Exposed**	**OR (95% C.I.)**	**% Cases Exposed**	**% Controls Exposed**	**OR (95% C.I.)**
Saw U.S. or Allied troops badly wounded or killed	39%	33%	1.31 (0.82 – 2.11)	35%	33%	1.06 (0.34 – 3.33)
Heard chemical alarms sounded	59%	53%	1.31 (0.83 – 2.07)	79%	67%	1.90 (0.57 – 6.32)
Directly involved in air combat	6%	5%	1.27 (0.48 – 3.38)	6%	0%	-

The relatively small sample size of Gulf War veterans in Study 2 (49 GWI Cases, 19 DC) precluded use of comprehensive multivariable modeling and subgroup analyses that might provide a more precise understanding of the contributions of individual and combinations of exposures in this group. However, further analyses of Study 2 exposure data indicated that including data collected on duration of exposure provided additional information on GWI risk in Study 2 participants. Findings that were significant or near-significant for associations between exposures of different duration and GWI are summarized in Table 39. Preliminary logistic regression models were also done to evaluate possible effects of confounding. However, due to the small number of Controls in the analyses, adjustments were made only for the effects of the most prominent risk factor, that is, being a regular smoker during deployment.

Bivariate analyses indicated that, in addition to being a regular smoker during deployment, use of PB for one week or longer was significantly associated with GWI (OR=3.14). In addition, significant and near-significant findings (based on chi square tests) were also associated with having contact with destroyed enemy vehicles for one week or longer (OR = 2.94, chi sq p=0.07), wearing uniforms treated with pesticides for one week or longer (OR = 4.53, chi sq p=0.05), and using pesticide cream or spray on the skin for one month or longer (OR = 7.50, chi sq p = 0.03). Adjustment for the effects of smoking during deployment did not substantially change the strength of identified associations with any of the other exposures in the table. However, subjecting the already small number in each exposure group to additional subsetting in these analyses did result in less statistical power and less stable estimates.

Table 39. Associations Between GWI and Self-Reported Exposures of Different Duration among Gulf War Veterans in Study 2

Exposure	**% Cases Exposed**	**% DC Exposed**	**OR (95% C.I.)** unadjusted	**OR (95% C.I.)** adjusted*
Regular smoker during deployment	63%	21%	**6.46 (1.86-22.46)**	--
Contact with destroyed enemy vehicles ≥ 1 week	53%	28%	2.94 (0.91 – 9.51)	2.50 (0.72 – 8.67)
Took NAPP (PB) pills ≥ 1 week	67%	39%	**3.14 (1.02 – 9.65)**	2.66 (0.81 – 8.75)
Wore uniform treated with pesticides ≥ 1 week	36%	11%	4.53 (0.93 – 22.14)	5.17 (0.98 – 27.19)
Used pesticide cream/spray on skin ≥ 1 month	31%	6%	7.50 (0.91 - 61.63)	8.41 (0.96 – 73.61)

*adjusted for being a regular smoker during deployment

As described for exposure analyses in Study 1, understanding potentially complex relationships between GWI and self-reported exposures in theater requires the careful, appropriate use of subgroup analyses, multivariable modeling, and evaluation of the effects of combinations of exposures that occurred in different subgroups. The small Study 2 sample of deployed Gulf War veterans, particularly the small number of DC, precluded comprehensive application of these procedures for this study. However, evaluation of risk associated with exposures of different duration indicated that use of PB for one week or longer is also associated with GWI in this sample. In addition, the near-significant

associations and high OR point estimates for wearing uniforms treated with pesticides and using pesticides on the skin for longer periods of time, suggest that these Gulf-war related exposures cannot be ruled out as possible contributors to GWI among veterans who served on the front lines. More definitive answers cannot be reached with this small sample.

What these exploratory evaluations do provide, though, is an indication that evaluation of the possible contribution of exposures in relation to GWI may not differ as substantially between Study 1 and Study 2 as had appeared based on preliminary analyses. That is, the most significant risk factors for GWI in Study 1 were use of PB and wearing uniforms treated with pesticides. In addition, Study 1 findings indicated that GWI risk associated with use of pesticide cream/spray on the skin was substantially elevated among veterans who also reported wearing uniforms treated with pesticides. Information on duration of individual exposures was not obtained in Study 1. As described, preliminary analyses from Study 2 implicate more prolonged use of PB as a risk factor for GWI, and also indicate that extended use of permethrin-treated uniforms and skin pesticides can not be ruled out as important risk factors for GWI.

Apparent differences in findings between the two studies are likely the result of both sample selection and sample size. The Study 2 sample would be expected to have experienced groups of Gulf War exposures related both to being in battlefield areas, and to serving with the Army - the two veteran subgroups identified as being at highest risk for GWI in Study 1. The Study 2 sample itself represents a subgroup of interest, as opposed to the broader sample included in Study 1. As shown in Study 1, associations between GWI and exposures appear to differ somewhat in different subgroups of veterans and investigations of exposure effects associated with veteran subgroups requires a sample size suitable for this task. Therefore, while Study 2 findings cannot be said, overall, to conflict with those of Study 1, neither can they be said to verify Study 1 results due to differences in both sample characteristics and the necessarily smaller Study 2 sample size. Study 2 was of necessity smaller than Study 1 because of the high costs of collection and analysis of physiological data.

The most significant difference between findings of the two studies, however, cannot be explained by the limited sample size in Study 2. The most significant risk factor for GWI in Study 2 was being a smoker during deployment. This association was not substantially diminished when it was included with other variables in the limited modeling analyses previously described. The large magnitude of this finding (OR=6.5), and its degree of statistical significance (p=0.002), given the relatively small Study 2 sample, indicate that smoking during deployment is an important risk factor for GWI among individuals in this unit who served at the battlefront.

It is possible to speculate about biological mechanisms that might plausibly underlie an association between GWI and smoking. Nicotine is a cholinergic agonist, and may have contributed directly to the development of GWI or interacted with other exposures that effect the cholinergic system such as low level exposure to nerve agents, PB, pesticides, and stress. All of the veterans in the Study 2 sample served with units identified by DOD models as having been in areas under the plume associated with demolition of the Khamisiyah weapons depot in March 1991. In addition, a relatively high proportion of veterans in the Study 2 sample report exposures to PB and pesticides.

But if smoking is an important contributor to the risk of GWI, it is not obvious why this association was not also identified in Study 1. The two most plausible explanations are (1) The

association with smoking identified in Study 2 was the result of a confounding or interactive relationship between smoking and an outside factor not adequately accounted for by the study (e.g. stress or a specific exposure) that was experienced by veterans in the Study 1 sample, but not commonly experienced in the more diverse Study 1 sample; and (2) that the Study 2 finding of an association between smoking and GWI was unique to the specific individuals who participated in Study 2 who, by chance, included an excess proportion of smokers among Cases but not among Controls. We cannot decide between these two explanations with the data available.

In summary, results of exposure analyses in both Study 1 and Study 2 indicate that veterans directly involved in ground combat are not at increased risk for GWI, but that exposure to pesticides and PB may be important risk factors for GWI. Additional details concerning findings that appear to differ between the two studies may be attributable to differences in the wartime experiences of veterans in the two samples, and to the lack of conclusive exposure findings from Study 2, owing to limited sample size and statistical power. However, the prominence of smoking as a risk factor in Study 2 but not in Study 1 cannot be readily explained using data collected for the two studies.

Relationship Between Study 1 and Study 2 BChE Genotype Distributions. Another related task was the examination of the BChE genotype distributions in Study 1 and Study 2. The reason for this task was that, after adjusting for the relative sizes of the two studies, it was unclear whether the frequencies of A, K and F BChE mutants are comparable in the two studies. An analysis of the BChE gene frequencies that gave a definitive answer to this question would provide useful information as to whether other observed differences between the populations in these two Studies could be ascribed to differences in BChE mutations. Differences in the distributions, if present, could have happened by chance, since the number of volunteers was smaller in Study 2 than in Study 1, or could reflect some unexplained association between carriers of BChE genotypes and the different demographics reflected in the populations in the two studies.

The chart below summarizes the information on the BChE genotypes in the two studies that has been presented previously for ease of visual comparison:

	U/U	U/K	K/K	U/AK	U/A	A/F	AK/F	All
Study 1	189	87	13	10	3	1	1	304
Study 2	59	25	3	1	0	0	0	88
Total	248	112	16	11	3	1	1	392

The distributions of BChE genotype proportions in the two studies were compared by means of contingency table analysis. Since 8 of the 14 cells had expected frequencies below 5, Fisher's exact test was used. The probability of the particular table configuration under the null hypothesis of non-homogeneous proportions is $p = 0.0012$. This supports the alternative hypothesis of homogeneous proportions in the two studies. In conclusion, the distribution of BChE genotypes in the two studies is statistically indistinguishable.

Further Evaluation of Previously-Identified Interactions Between Gulf War-Related Exposures and BChE Genotype, in Relation to Risk for GWI. With the knowledge that the sampling

of BChE genotypes in the two studies was comparable, which adds confidence to our sampling strategy, and with the considerations related to confounding discussed above, it is possible to conduct further evaluations regarding previously-identified interactions between BChE genotype and war-related exposures.

It is important to note that while data on war exposures may be subject to uncertainties of recall bias, data on BChE genotype is not driven by such a bias. Consequently, data on the interaction between BChE genotype and the war-related exposures is likewise not driven solely by such a bias. The reasons for this are that the BChE genotype is a stable molecular property of the individual, which in our study was determined by an objective test. Moreover, that test was conducted after the veterans had provided information on wartime exposures. Veterans were not aware of their BChE genotype prior to providing information on exposures. Therefore, in the Case of the highly-elevated risks for GWI we observed as an interaction between the condition of being carriers for low-velocity variants of BChE and reporting certain wartime exposures, it was not possible for the variant carriers of the BChE mutations to have consciously or unconsciously overstated their wartime-related exposures.

As previously described, Gulf War veterans identified as BChE variants were found to be at significantly increased risk for developing GWI if they reported a number of specific exposures during the war. The greatest interaction between exposure and BChE variant status occurred with self-reported use of PB during deployment, which was associated with an OR of 2.68 (95% C.I. 1.62-4.44) overall among Gulf veterans who were not BChE variants, but a significant, highly-elevated OR of 40.0 (95% C.I. 3.58-447.0) among BChE variants.

Significant, but less dramatic interactions were also identified between BChE variant status and participation in ground combat, seeing Iraqi or civilian casualties, contact with prisoners of war, contact with dead animals, frequently having less than 4 hours sleep in a 24-hour period and, potentially, with receipt of vaccines during deployment. These findings were based on a small variant sample size, and therefore must be interpreted with caution. Still, they provide a testable hypothesis that, if verified in future studies, may represent an important step forward in understanding possible associations of wartime exposures and the development of chronic health problems following the Gulf War. Therefore, it was important to more carefully evaluate these findings.

Multivariable Modeling Among BChE Variants. It is common for misconceptions about relationships between GWI and Gulf War-related exposures to arise from errors introduced by confounding. Additional analyses were performed to determine whether Gulf War exposures that appeared to interact with BChE variant status were true risk factors or merely the consequence of confounding by other variables. Due to the small number of BChE variants in the sample, a comprehensive evaluation of this question, using modeling procedures that control for the effects of all variables of interest simultaneously, was not possible. A more limited evaluation of possible confounding was undertaken by controlling for the effects of PB exposure in assessing the effects of other risk factors that had appeared to interact with BChE variant status, and is summarized in Table 40 below.

Table 40. Association of Gulf War Illness with Exposures Among BChE Variants Controlling for Effects of PB

Exposure	% Cases Exposed	% Controls Exposed	Unadjusted OR (95% C.I.)	Adjusted OR (95% C.I.)
Took NAPP (PB) pills	92%	23%	**40.0 (3.58 – 447.0)**	**40.0 (3.58 – 447.0)**
Saw Iraqis or civilians badly wounded or killed	86%	29%	**15.0 (2.26 – 99.64)**	5.38 (0.51-56.6)
Had contact with prisoners of war	86%	29%	**15.0 (2.26 – 99.64)**	2.36 (0.17-32.99)
Saw/had contact with dead animals	86%	14%	**36.0 (4.33 – 299.0)**	9.09 (0.77-107.5)
Directly involved in ground combat	57%	14%	**8.00 (1.27 – 50.04)**	0.70 (0.05-10.01)

These evaluations were possible for four of the variables of interest, but were not possible for two of the variables (shots received in the arm, frequently having less than 4 hours sleep in 24 hours) due to zero cells in those subgroups. As shown, results indicate that controlling for the effects of PB significantly reduced associations between GWI and each of the other variables evaluated. This suggests that apparent interactions between BChE genotype and seeing civilian casualties, contact with prisoners of war, contact with dead animals, and participation in ground combat all were due, in large part, to confounding by PB. In contrast, associations between PB and GWI remained elevated when controlling for the other individual exposures evaluated. This indicates that a significant excess risk for GWI occurred among BChE variants who were exposed to PB, but not among BChE variants who experienced the other exposures evaluated.

Is Interaction Between PB and BChE Variant Status Dependent on the GWI Case Definition Used? Because the interaction identified between PB and BChE variant status is a novel and important finding, it is reasonable to ask whether it represents an anomaly related to the GWI Case definition used in this study, the KVP Case definition developed as part of the Kansas Persian Gulf War Veterans Health Project by Dr. Steele[17]. Another GWI-related Case definition used in several studies is the definition for "chronic multisymptom illness" or CMI, developed by Fukuda and colleagues[14], often referred to as the "CDC" Case definition. The CDC Case definition is generally less conservative than the KVP Case definition. That is, individuals meeting criteria for this Case definition are required to have fewer and less severe symptoms than those meeting criteria for the KVP Case definition.

As shown in Table 41 below, the interaction between BChE variant status and PB exposure for GWI was not a unique result of the Case definition used in the present study. Interaction between PB and BChE variant status was also found when the CDC Case definition for CMI was used to characterize ill health in Gulf War veterans. As expected, the observed effect was less pronounced than that observed with the KVP Case definition. However, the elevated risk identified for CMI, a relatively nonspecific Case definition for ill health in Gulf veterans, indicates that the observed interaction between PB and BChE variant status in association with GWI is a robust finding.

Table 41. Interaction Between PB Exposure and BChE Variant Status Comparison of Results Using KVP and CDC Case Definitions

	Nonvariants Only			BChE Variants Only		
	% Cases Exposed	% Controls Exposed	OR (95% C.I.)	% Cases Exposed	% Controls Exposed	OR (95% C.I.)
Kansas GWI Case Definition	69%	46%	**2.68 (1.62 – 4.44)**	92%	23%	**40.0 (3.58 – 447.0)**
CDC Multisymptom Illness Case Definition	62%	49%	**1.73 (1.05 – 2.84)**	78%	22%	**11.37 (1.65 – 78.38)**

2.5.2 Molecular Biology and Biochemistry.

Genotypic and phenotypic analysis of PON1. Serum Paraoxonase [PON1, EC 3.1.8.1], initially characterized as an organophosphate hydrolase, is a high-density lipoprotein-associated serum enzyme. Two reports (one from the U.S.[63], one from the U.K.[64]), report associations between Gulf War veteran Case status and PON1 velocity (but not genotype) in serum. The U.S. study had a very small sample size, while the U.K. study may be subject to methodological flaws and did not examine serum cholinergic enzymes. A subsequent study from the U.K., published while our supplementary studies were in progress, again reported an association between reduced PON1 velocity and Case status in GWI.[65]

Genotypic analysis (and not just phenotypic determination of enzyme velocity) is important for PON1 because two polymorphisms are known in the coding region, which affect substrate specificity. In addition, five polymorphisms in the promoter region have been identified. Of all of these mutations, the most important polymorphism is the Q192R polymorphism (hereby denoted Q or R), which determines catalytic efficiency of hydrolysis of some substrates, while some promoter polymorphisms, like C-108T, help regulate the level of expression of PON1 and hence its overall velocity in serum. For this reason we undertook to re-contact veterans who had participated in Study 2, and requested their permission to have leftover samples in our freezer assayed for PON1 Q192R genotype and phenotype. Phenotype was determined by assaying PON1 enzyme velocity under 3 substrates (phenyl acetate, diazoxon and paraoxon).

PON1 Assay Methods. All assays were done in triplicate and repeated if there was a large variation in the readings. The assay for phenyl acetate activity used 2.5 mM phenyl acetate in 50mM Tris Cl, pH = 8.0, 1.0 mM $CaCl_2$ buffer [66]. The human serum for phenyl acetate assays was diluted 1:40 in buffer. Five microliters of diluted serum was pipetted into each well and 200 ml phenyl acetate in buffer was added. The absorbance was recorded at 270 nm for 3 minutes. The extinction coefficient in a 96-well plate is 38% of what it is in a 1ml cuvette leaving a value of $0.4978 mM^{-1}cm^{-1}$.

It should be noted that phenyl acetate is also a substrate for BChE, and in principle BChE activity could "contaminate" these measurements. Control experiments in the presence of 250 mM EDTA (which inhibits PON1 activity), 2 mM iso-OMPA (which inhibits BChE), or combined EDTA +

iso-OMPA (to determine a background rate) established that under the conditions of this assay BChE activity accounts for about 1% of the phenyl acetate hydrolysis, which is a negligible amount. Thus under the conditions of these assays any potential contribution of BChE can be safely neglected, and the phenyl acetate hydrolysis can be interpreted to arise from PON1.

The assays for diazoxon and paraoxon followed the protocols of Richter and Furlong[67]. The diazoxon assay used 0.1M diazoxon in 0.1 M Tris Cl, pH = 8.5, 2 M NaCl, 2 mM $CaCl_2$. Prior to assay human serum was diluted 1:5 in buffer. Six microliters of diluted serum was pipetted into each well and 200 ml diazoxon in buffer was added. Absorbance at 270 nm for recorded for 3 minutes. The extinction coefficient adjusted for the 96-well plate is 1.15 $mM^{-1}cm^{-1}$.

The assay for paraoxon used 1.2 mM paraoxon in 0.1 M Tris Cl, pH = 8.5, 2 M NaCl, 2 mM $CaCl_2$. Serum was not diluted for this assay. Five microliters of serum was pipetted per well and 200 ml paraoxon in buffer was added. Absorbance was read at 405 nm for 3 minutes. The adjusted extinction coefficient was 6.84 $mM^{-1}cm^{-1}$. Both paraoxon and diazoxon were purchased from Chem Service, West Chester PA, with guaranteed purity of greater than 98%.

PON1 Results. We were able to re-contact and obtain permission to re-assay samples from 91 of the 116 volunteers who originally participated in Study 2 (78.4%). Twenty-four of the original 116 volunteers were lost to follow-up, and only one declined to have his/her stored sample assayed for PON1. For one of the volunteers, PON1 status could not be determined because the samples had initially been erroneously collected in vaccutainer tubes that contained anticoagulant, which interferes with the assays. Although assayed for PON1, for subsequent analyses two nondeployed Cases, which had been excluded for the physiological measures were also excluded in examinations of PON1, Case status, and physiology.

The Case/Control breakdown of the volunteers who agreed to have their stored samples tested for PON1 was similar. There were 49 Cases who agreed to the testing, and 44 Controls.

The results of PON1 phenotype (velocity) testing and genotyping are summarized in Table 42. The frequencies of the Q allele (0.7) and of the R allele (0.3) in this population are consistent with the observed demographics of our Study 2 population (Table 4 above), and the observed distribution of Q and R alleles in different ethnic groups.[68]

Table 42. PON1 velocity using phenyl acetate, diazoxon and paraoxon and PON1 genotype

Volunteer	paraoxon u/ml	phenyl acetate u/mL	diazoxon u/ml	Genotype
1001	0.1685	43.0778	5.4701	QQ
1014	0.2216	64.5832	6.3752	QQ
1025	0.2479	64.6633	9.4508	QQ
1067	0.2046	61.0592	6.0738	QQ
1077	0.2551	66.4237	6.8369	QQ
1095	0.2252	72.8251	8.8499	QQ

Volunteer	paraoxon u/ml	phenyl acetate u/mL	diazoxon u/ml	Genotype
1136	0.1728	57.6395	5.5105	QQ
1169	0.2889	81.1512	9.8462	QQ
1190	1.3338	74.2372	5.5731	RR
1248	0.1443	51.4052	6.4987	QQ
1271	0.1696	67.2111	5.9195	QQ
1288	0.0963	35.3434	3.6547	QQ
1401	0.3534	108.7734	6.9068	QQ
1404	0.6522	76.1359	3.7580	QR
1468	0.2191	86.9375	8.2110	QQ
1603	0.2354	59.3878	10.5644	QQ
1623	0.1344	49.3176	5.5165	QQ
1632	0.0847	27.7825	3.3323	QQ
2092	0.3398	85.6757	6.1136	QQ
2102	0.8404	64.2933	3.5299	QR
2142	0.1708	74.0823	7.8337	QQ
2416	0.8842	107.5698	9.0065	QR
2564	0.0898	32.8209	1.1855	QQ
2600	0.8272	53.6520	3.8371	QR
4102	0.5527	76.6916	5.6196	QR
4104	0.7898	75.6736	5.0549	QR
4110	0.2514	74.5260	9.5829	QQ
4115	0.2834	126.1870	13.1118	QQ
4119	0.4843	70.1135	5.0219	QR
4120	0.5872	85.2397	6.9931	QR
4123	0.3209	108.5395	8.2565	QQ
4124	0.8287	110.7347	10.2202	QR
4125	0.4721	137.0830	18.4280	QQ
4128	0.4851	112.7883	17.6815	QQ
4129	1.0643	122.6257	10.5326	QR
4130	0.2725	89.9025	7.4981	QQ
4135	0.3497	88.3980	5.4782	QQ
4136	0.6794	79.3162	1.2617	QR
4139	0.8839	100.0254	4.8825	QR
4142	1.4622	107.3513	2.8595	RR
4143	1.3368	128.7644	4.8588	RR
4144	0.2504	104.0524	7.9085	QQ
4146	2.0015	99.4291	3.6810	RR
4147	1.0079	124.9121	10.5957	QR
4148	0.4821	119.5190	14.1616	QQ
4150	0.4394	102.9784	8.6304	QQ
4153	0.1667	83.9153	7.6291	QQ
4158	0.2963	123.1737	12.3993	QQ
4159	0.2900	91.8111	14.7477	QQ
4163	0.8842	89.1700	10.0676	QR

Volunteer	paraoxon u/ml	phenyl acetate u/mL	diazoxon u/ml	Genotype
4304	1.0489	114.1380	7.4808	QR
4317	0.1819	68.0808	6.6530	QQ
4348	0.9990	78.3696	3.9632	QR
4365	1.6817	99.1183	5.0640	RR
4388	1.1552	108.8250	4.3961	QR
4396	1.0767	104.8694	6.3532	QR
4404	0.8026	101.5332	4.5022	QR
4410	1.0439	108.8766	9.9658	QR
4435	0.8696	121.5879	10.1849	QR
4436	0.2432	105.0869	9.5912	QQ
4437	0.9636	104.8584	6.7177	QR
4442	0.1861	62.6087	10.2038	QQ
4448	1.8367	182.7229	6.5320	RR
4454	1.1489	81.0645	7.5236	QR
4467	0.3784	103.1640	8.6233	QQ
4480	0.3547	135.1250	17.2725	QQ
4482	1.8299	95.5438	8.3588	RR
4506	1.5400	121.8811	2.8400	RR
4526	0.2218	101.4080	7.8828	QQ
4530	0.8076	126.4561	10.1364	QR
4534	0.1941	104.9661	10.2235	QQ
4544	1.2200	99.7805	10.7986	QR
4549	0.1874	92.3954	7.3105	QQ
4551	0.2229	97.5403	7.6981	QQ
4552	0.3851	101.9703	5.6577	QQ
4553	0.2637	118.3099	7.9921	QQ
4554	1.3011	89.2469	4.7065	RR
4560	1.3702	84.2524	9.7294	RR
4565	1.4428	111.1784	5.5774	RR
4572	0.3444	116.4617	12.0909	QQ
4581	0.2357	98.1860	8.7349	QQ
5103	0.1034	55.2828	2.6129	QQ
5104	0.3564	109.0007	10.4772	QQ
5106	1.4598	90.6000	9.5508	RR
5107	1.8578	113.2397	5.8688	RR
5108	0.2855	123.4273	7.3926	QQ
5372	0.6576	86.6157	7.8614	QR
5374	0.2074	99.3885	9.7997	QQ
5397	0.0298	11.4704	1.0578	QQ
5398	1.4188	97.8774	3.2099	RR
5410	0.2753	83.3201	6.6967	QQ

The clear relationship between genotype and substrate preference is shown graphically in Figures 14 and 15, and summarized in Table 43.

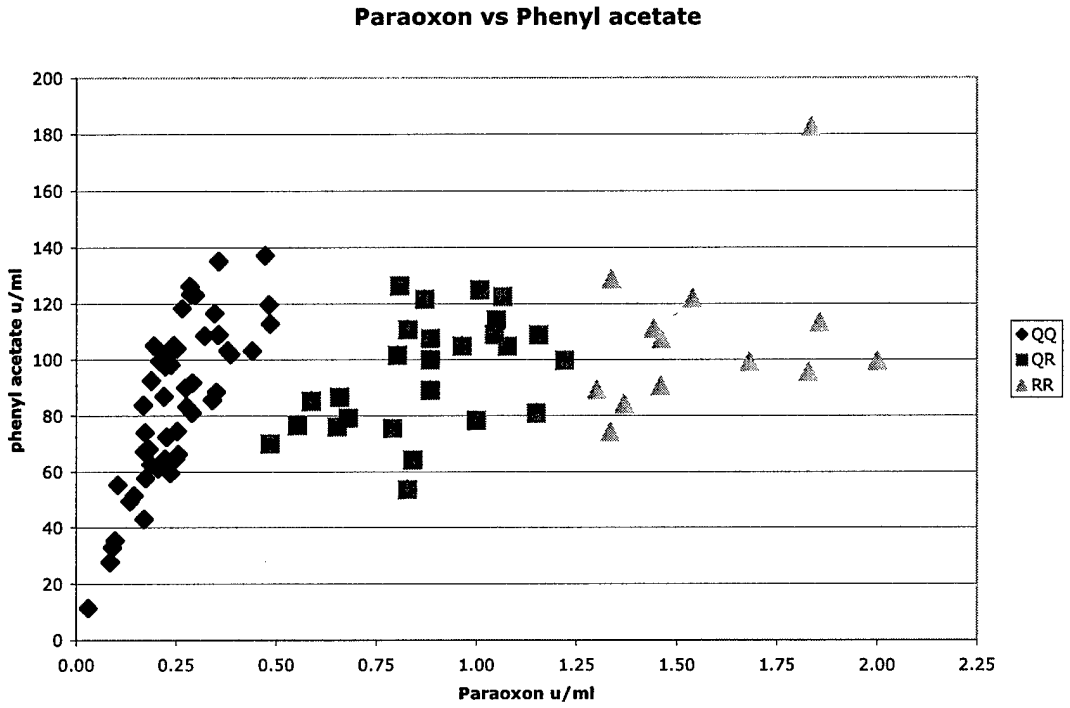


Figure 14. This figure illustrates PON1 velocity, plotting paraoxon against phenyl acetate, stratified by genotype. The diamonds (dark blue) represent QQ homozygotes, the squares (magenta) represent QR heterozygotes, and the triangles (light blue) represent RR homozygotes.

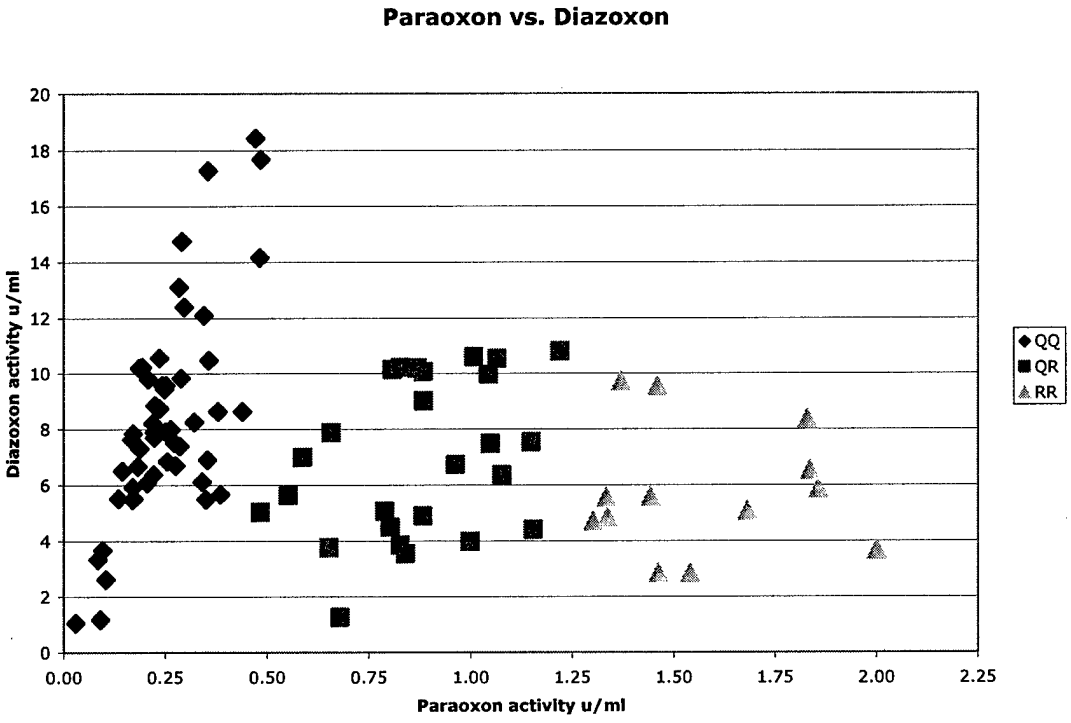


Figure 15. This figure illustrates PON1 velocity, plotting paraoxon against diazoxon, stratified by genotype. The diamonds (dark blue) represent QQ homozygotes, the squares (magenta) represent QR heterozygotes, and the triangles (light blue) represent RR homozygotes.

Table 43. Association of PON1 Activity Levels with Q192R Genotype (n = 88)

	PON1 Activity Levels (u/ml)					
Substrates	Paraoxon		Phenyl acetate		Diazoxon	
	mean	(s.e.)	mean	(s.e.)	mean	(s.e.)
QQ Genotype	0.2593	(0.0139)	87.214	(3.8961)	8.6523	(0.5023)
QR Genotype	0.8841	(0.0387)	95.461	(4.0768)	6.8962	(0.5700)
RR Genotype	1.5624	(0.0623)	106.82	(7.0298)	5.6007	(0.6084)
Pairwise comparisons*						
QQ vs. QR	$p < 0.001$		$p = 0.187$		$p = 0.034$	
QQ vs. RR	$p < 0.001$		$p = 0.020$		$p = 0.003$	
QR vs. RR	$p < 0.001$		$p = 0.141$		$p = 0.153$	

* t tests

PON1 genotype at the Q192R locus and Case/Control Status. Table 44 shows the proportions of GWI Cases, DC, and NDC with QQ, QR, and RR genotypes. Note that although there is a lower proportion of RRs in the DC group, PON1 genotype distributions do not differ significantly between GWI Cases and DC, nor between the 2 Control groups. Using a chi squared test, statistical comparison of the proportion of RRs in the DC and NDC groups does not reach statistical significance, with a p = 0.075. These results differ from those reported by Haley and co-workers[63], who found that GWI Cases had a higher frequency of carriers of the R allele.

Table 44. Distribution of PON1 Genotypes in Study 2 GWI Cases and Controls

					Pairwise Comparisons of PON1 Genotype Distribution		
	Cases (n=40)	**DC** (n=18)	**NDC** (n=14)	**All Controls** (n=32)	**Cases vs. DC***	**Cases vs. All Controls***	**DC vs. NDC***
QQ Genotype	50%	61%	43%	53%	p = 0.287	p = 0.759	p = 0.200
QR Genotype	28%	33%	29%	31%			
RR Genotype	22%	6%	29%	16%			
% with R allele (QR+RR)	50%	39%	57%	47%	p = 0.433	p = 0.792	p = 0.305
% with RR genotype	22%	6%	29%	16%	p = 0.114	p = 0.464	p = 0.075

* Chi squared tests

Table 45 compares mean PON1 velocity levels among GWI Cases, DC, and NDC for each of the 3 substrates. PON1 activity in paraoxon was significantly *lower* in DC than in Cases (p=0.04). Velocity levels in paraoxon were similar in Cases and NCD. Using phenyl acetate as a substrate, mean values for DC and NDC were similar.

Table 45. PON1 Velocity in 3 substrates Case/Control Analysis

	Mean Activity Levels (u/ml)				pairwise comparisons		
Substrate	**Cases** (n=40)	**DC** (n=18)	**NDC** (n=15)	**All Controls** (n=33)	**Cases* vs. DC**	**Cases* vs. All Controls**	**DC* vs. NDC**
Paraoxon	0.7435	0.5031	0.8190	0.6467	p = 0.044	p = 0.436	p = 0.090
Phenyl acetate	101.03	93.53	91.75	92.72	p = 0.282	p = 0.158	p = 0.861
Diazoxon	7.997	8.850	6.503	7.783	p = 0.489	p = 0.802	p = 0.085

* t tests

Haley and colleagues[63] reported that low activity of the PON1 Q allele distinguished GWI Cases from Controls better than PON1 genotype by itself or the activity levels of the type R allele, or total paraoxonase. While we did not find corresponding results with respect to the Q allele, similar to our results, Haley, et al.[63] also found higher PON1 activity in ill veterans, when activity was measured with paraoxon as substrate. In contrast, the recent paper by Hotopf and colleagues[65] reports that GWI Cases had low PON1 activity, measured with paraoxon. That is an opposite finding to that observed by Haley's group or ours. The difference reported between GWI Cases and healthy veterans was 15%, which is a small difference. They reported a bigger difference when they compared veterans who spent time in the Persian Gulf to veterans who never deployed there; in their data set deployment rather than Case status made the bigger difference.

In conclusion, our data on PON1 velocity when using paraoxon as the substrate and Case/Control status is in agreement with the report of Haley et al[63]. However, we did not see, in a larger sample size than that present in his report, an association of Case/Control status with PON1 genotype, nor an association of Case/Control status with PON1 velocity using other substrates.

2.5.3 Autonomic Nervous System Physiology

Spectral analyses of the time-difference between R-wave and pulse-pressure wave: A number of the significant findings in Study 2 involved alterations in HRV, and some of the affected HRV measures, like spectral Low Frequency power are believed by some to reflect predominantly, but not exclusively, sympathetic nervous system activity. In this supplementary task we sought to further confirm or deny specific sympathetic nervous system changes to the responses to ANS stressors using a novel measure of sympathetic activity.

The average time difference between the peak of the R-wave of the ECG and the peak of the pressure wave is a measure of pulse transit time, often used clinically as a surrogate diagnostic for arterial stiffening due to artherosclerosis. However, the beat-to-beat difference between these two events is not constant, and the fluctuations of these beat-to-beat differences around the mean reflect rhythmic arterial relaxation and stiffening due to sympathetic activity involved in BP control. This is purely a sympathetically-mediated phenomenon, since the parasympathetic nervous system does not innervate the systemic vasculature, except for the external genitalia and some parts of the cerebral vasculature, and those two specialized vascular beds do not contribute significantly to pulse-transit time. If the sympathetic activity reflected in HRV were identical to pulse pressure variability, then the time difference between the R-wave and the peak of the pressure wave would be constant and that would in turn lead to a flat spectrum with a value of zero. We and others have found that this is not the Case, and that useful information can be obtained by a spectral analysis of the time series of differences between these two events.

We conducted this analysis with data colleted in Study 2. We continuously recorded the ECG and non-invasive arterial tonometry. With the latter technique the full waveform of the radial artery BP was obtained on a beat-to-beat basis, with a time resolution of 0.004 msec, which is the same time resolution at which we had the digitized the ECG. Our custom software identified the times of occurrence of the peaks of the pressure wave, which were used in SBP determinations.

To gain better insights into the differences in sympathetic activity between Cases and Controls, we calculated the time difference between the R wave of the ECG and the arrival of the pulse at the radial artery. These data were first converted to a time series with equal intervals by means of a spline interpolation. The time series was subsequently detrended, and the detrended time series subjected to a FFT. If in fact Cases have more sympathetic activity than Controls, the pulse pressure wave should arrive at the radial artery more quickly for Cases than Controls. The time difference between the radial artery pulse and the R wave should therefore be less, and the FFT should show a peak at a higher frequency for Cases.

The frequency of the highest peak at or above 0.10 Hz was submitted to multivariate ANOVA in which Groups (Cases, DC, NDC) was the independent factor and Periods (initial baseline, tilt baseline, second five min of head-up tilt, third five min of head-up tilt, min 2-5 of return to the horizontal, and minutes 6-10 of return to horizontal. The initial period of head-up tilt, and the initial minute after returning to the horizontal position were not included, as visual inspection of the FFTs revealed that they were not stable. We restricted our analyses to head-up tilt and recovery from tilt because the tilt task best discriminated between Cases and Controls in our original HRV analyses. A second ANOVA, using the same format, was used to compare the variant and nonvariant participants as determined by previous BChE genetic analyses.

Case/Control Analysis: Frequency of the highest FFT peak differed significantly as a function of group ($F = 2.46$, df 12, 96, $p = .008$). The group differences occurred primarily in the second and third 5-min periods of head-up tilt and min 2-5 of recovery. The results are shown in Table 46:

Table 46. Case/Control Analysis of Pressure Wave - R wave Differences.

Highest FFT Peak in the Difference Spectrum, in Hz.

	Up 2 – Mean (SD)	Up 3 – Mean (SD)	Down 3 – Mean (SD)
	F=4.93, df 2, 53, p=.011	F=6.095, df 2, 53, p=.004	F=3.762, df 2, 53, p=.030
Cases	.264 (.077)	.272 (.071)	.283 (.074)
DC	.186 (.085)	.197 (.079)	.239 (.081)
NDC	.260 (.083)	.282 (.068)	.214 (.085)

Differences in periods interacted with group ($F = 2.54$, df 10, 98, $p = .009$). The results are shown in Table 47. During head-up tilt, Cases showed higher frequency peaks than did the DC. The NDC did not differ from the Cases. Recovery from tilt was associated with less marked group differences, with the NDC group showing the lowest frequency.

Table 47. Case/Control Analysis of Pressure Wave - R wave Differences Across all Periods

	Mean (SD)		
	Cases	DC	NDC
Baseline	.250 (.058)	.248 (.052)	.213 (.059)
Tilt Baseline	.269 (.074)	.242 (.087)	.213 (.074)
Up2	.264 (.077)	.186 (.085)	.260 (.083)
Up3	.272 (.071)	.197 (.079)	.282 (.068)
Down2	.265 (.084)	.264 (.105)	.250 (.071)
Down3	.283 (.074)	.239 (.081)	.214 (.085)

Results of the Case/Control analysis support the hypothesis that Cases would have higher frequency than DC. The results for the NDC group are not consistent with this hypothesis, and are hard to explain. According to our hypothesis, NDC should be less sympathetically activated, and thus, would be expected to show lower frequency than the other two groups, but they did not. We did not see frequency-domain HRV differences in reactivity between groups during the tilt segment of the battery, although Cases had lower Power than either Control group. For some time domain measures, the NDC group was greater at baseline than either Cases or DC, but those time domain measures (like %NN or rMSSD) are not directly relatable to either sympathetic or parasympathetic activation.

Variant/nonvariant Analyses. In these analyses, we asked whether BChE genotype had an effect on sympathetic activity, as reflected in the position of the peaks of the spectra of the differences of the R wave peaks and the pressure waves. This was a natural question to ask, since in Study 2 we had seen interactions between BChE genotype and some physiological endpoints that may be sympathetically-mediated. Mean frequency was higher for the nonvariant group than for the variant group when examined across all six periods, ($F = 2.52$, df 6, 67, $p = .03$), and the difference was significant for both the baseline period ($p = .001$) and the second 5 min of head-up tilt ($p < .03$). Values are shown in Table 48.

Table 48. Mean Frequency (Hz) for Variant and Nonvariant groups

	Mean (SD)	
	Nonvariant	Variant
Baseline	.244 (.058)	.191 (.047)
Tilt baseline	.253 (.079)	.220 (.064)
Up2	.244 (.087)	.221 (.084)
Up3	.258 (.078)	.214 (.062)
Down2	.265 (.085)	.230 (.071)
Down3	.258 (.082)	.242 (.064)

In conclusion, application of the technique of FFT to the time difference between the R wave and the peak of the pressure wave provided useful information, although this information was not as clear-cut as anticipated in the hypotheses. This is a novel technique, so surprises are to be expected. The frequency of the largest peak in the FFT of time difference was informative with respect to Case/Control differences and also with respect to variant/nonvariant differences. The Case/Control analyses supported the hypothesis that Cases would have higher frequency than DC, which was expected from the higher presumed sympathetic/adrenergic tone in this group. The results with the NDC were not expected and we have no explanation for them. The variant/nonvariant analyses were informative, and complement the differences we had already found between these two groups in other baseline parameters as a function of BChE genotype.

Efforts to Relate changes in mean HR, BP and HRV during tasks to exposures. As part of the statistical analyses of Study 2 results, we related Case/Control status and BChE genotype status to changes in physiological parameters. In this additional task, we related the physiological variables to dichotomous exposure variables by subgroup analyses, stratified by Case/Control status to avoid the colinearity in the variables. NDC veterans were not included in these analyses, as there was no in-theater exposure data for this group.

Separate ANOVAs were performed for three exposure parameters (taking PB for at least a week; applying pesticide cream or spray to skin for at least a month; wearing a uniform that had been sprayed with pesticide for at least a month), and to a combination of pesticide exposure and PB. Four time periods served as repeated measures (baseline, initial head up tilt, the last 5 min of head up tilt, and the initial response to returning to the horizontal position). HR, SBP, and ABS LF HRV were the dependent variables.

As expected from previous analyses, the four time periods differed significantly for each of the exposure variables and for all of the dependent variables. No significant differences between exposed and non-exposed subgroups were found for either Cases or DC, indicating that exposure status had little effect on the physiological results for either group.

Efforts to Relate the stress ratings to the physiological changes observed during the emotional stress task. In previous analyses, Cases had higher subjective ratings of stress associated with recalling and describing a stressful event than either of the two Control groups. In addition, these tasks produced differences between Cases and Controls in some physiologic variables. In this task, we related the stress ratings to the physiological changes observed during the emotional stress task to determine whether we might be able to identify subgroups at greater risk for maladaptive physiological responses to emotional stress.

Multiple regression analysis was used to address this issue. Self-reported stress ratings during the recall of a stressful event served as the dependent variable; Case status (Case vs. Control) and genetic status (variant vs. nonvariant) as well as the interaction between Case status and genetic status were the predictors. Multiple regressions were calculated for mean HR, mean BP, Mean DBP, SDNN, and SDSD. The physiological values were defined as the baseline value less the task value divided by 100 (% change).

No statistically significant predictors were identified. The relationship between physiological differences during recall of a stressful event and subjective ratings of stress during the recall did not appear to depend on Case status, variant status, or their interaction.

Mathematical analyses of tilt and tilt recovery data. This task consisted of attempting to assign each individual's tilt data to "functional" and "dysfunctional" categories based on patterns of mean HR, SBP and DBP changes during orthostatic stress. It was hoped that sophisticated methods of analysis (e.g., Pearson correlations between the time-series of SBP and DBP against HR during tilt) that may optimize the differences between Cases and Controls could provide a better understanding of the differences between the groups that result in the significant changes we observed during tilt and recovery from tilt. Several such methods were used with the data available from Study 2, using as the units of analysis the various periods (e.g., Tilt baseline, minutes 1-5 after Up Tilt, etc) that had previously been used for analyses of the HRV time series. Attempts were made to use the results of those computations to obtain functional/dysfunctional classifications. None of the attempts resulted in a small set of non-overlapping categories. Consequently, no useful results were obtained from these supplementary analyses, beyond those discussed elsewhere in this report.

Section 3.
Key Research Accomplishments

3.1 Study 1

- Recruitment goals were successfully completed. 160 Cases and 144 Controls participated in the study (target values were 150 each).
- All genetic testing was completed for the volunteers who took part in Study 1.
- Statistical analysis was completed.
- The original form of the hypothesis being tested, that the low-velocity A, K and F mutations of BChE would be present more often in the Cases than in the Controls, was not supported. However, some related, statistically-significant findings emerged.
- A significant association between the K/K genotype and Case status was found.
- Strong and significant associations between K/K genotype and reported sleep loss, and between Case status and reported exposures during deployment were also found.
- The above associations do not correlate with the enzyme velocity, and are not present in the volunteers who only have one copy of the K allele, regardless of whether the other copy has a normal velocity (U) or has one or more low-velocity mutations (A, F, or AK).
- Discovered a new, naturally-occurring mutation of human BChE, Asp70His.

3.2 Study 2

- An autonomic nervous system reactivity test battery was developed and tested.
- Recruitment and testing of 49 Case veterans who met criteria for Gulf War Illness, 19 DC volunteers from the same units, 23 NCD veterans who served in the U.S. Army during the Gulf War, but were not deployed to the Persian Gulf, and 23 veterans from Study 1 who were identified as having mutations of BChE were completed.
- Off-line processing and statistical analyses of physiological data were performed, and demonstrated that the test battery produced the expected physiological changes with a high degree of consistency, and that:
    - Cases had higher mean BP and DBP than controls, supporting the hypothesis that GWI was associated with greater ANS reactivity.
    - Cases and Controls differed significantly during all battery tasks other than Valsalva, Hand-grip and Mental Arithmetic.
    - Cases showed less physiological response to head-up tilt than either the DC or NDC groups

- The startle response was smaller for Cases than for either Control group.
- Participants who were carriers of low-velocity mutations of BChE (variants) differed physiologically from nonvariant participants, exhibiting a lower HR, greater HRV, and greater response to startle.
- There were complex interactions between Case-Control and BChE variant status. Endpoint responses (mean HR, BP parameters, HRV parameters) to stressors differed as a function of both Case-Control status and variant group status.

3.3 Additional Tasks

- Completed further multivariable analyses of the relationship between various exposures and GWI, which revealed associations with both pesticides and PB.
- Examined the possible role of confounding in the observed associations.
- Identified an interaction between BChE variant status and exposure to PB as a strong risk factor for GWI.
- Demonstrated that our finding with respect to the BChE variant/PB exposure interaction was robust whether the CDC or KVP Case definition was used.
- Tested whether PON1 genotype or phenotype (with 3 substrates) correlated with Case/Control status. Demonstrated higher PON1 velocity in Cases with paraoxon as substrate.
- Developed methods to further explore sympathetic nervous system changes in the responses to ANS stressors. The comparison between Cases and Controls showed higher sympathetic tone for Cases than for DC; the NDC, however, did not show lower sympathetic activity. Furthermore, the nonvariant group had more sympathetic activity than the variant group.

Section 4.
Reportable Outcomes

4.1 Published Papers

Boeck, A., D. L. Fry, A. Sastre, and O. Lockridge, "Naturally Occurring Mutation, Asp70His, in Human Butyrylcholinesterase," *Annals of Clinical Biochemistry*, 39:154-156 (2002).

A number of other manuscripts are under peer-review or in preparation.

4.2 List of Personnel

- Sastre, Antonio
- Cook, Mary R.
- Graham, Charles
- Gerkovich, Mary M.
- Lockridge, Oksama
- Steele, Lea
- Bauer, Karin M.
- Riffle, Donald W.
- Hoffman, Steven J.
- Norman, Jeff D.
- Hatch, Sarah J.
- Spalding, C. L.
- Jones, Diane W.
- Dozier, Deborah I.
- Horn, Linda D.
- Hackman, Jeffrey L.
- Molen, A. M.
- Peterson, Rebecca C.
- Whitson, Kristine
- Wilson, Angela M.
- Decker, Emmett K.
- Evans, Stevens M.
- Faulkner, David N.
- Aldenderfer, Richard W.
- Racela, Lourdes, A.

Section 5. Conclusions

Study 1 re-examined a preliminary study previously conducted by Dr. Oksana Lockridge. In a self-selected volunteer sample and self-described GWI/healthy status, Dr. Lockridge found that heterozygous carriers for the A and F variants of BChE were found in a greater proportion (9:1-10:1) of veterans with symptoms of GWI than in veterans without such symptoms[18]. There was also a much weaker association with homozygous carriers for the K mutation. In our first study we re-tested the hypothesis that emerged from Dr. Lockridge's preliminary work using the methods of molecular biology on a well-defined population of GWV, with the benefit of a validated and structured symptom and history questionnaire. The results of our re-examination failed to confirm the greater proportion of A and/or F variants among the Cases than among the Controls. However, we did observe some statistically-significant associations between carriers of the K/K genotype and symptomatology associated with sleep deprivation, respiratory and gastrointestinal problems, as well as other conditions. These new associations must be considered hypothesis-generating, to be further tested in future studies. In the course of this study we also identified a new naturally-occurring mutation, Asp70His, in human BChE.

The results of Study 1 helped clear up a potentially misleading clue in the search for causes or factors associated with GWI. These results make clear that BChE genotype, *by itself*, does not determine or strongly influence Case/Control status. Nonetheless, the results obtained in Study 1, with the benefit of the results obtained in Study 2, have helped uncover some complex interactions of BChE genotype with other factors. The results indicate that BChE genotype can play an important role in baseline ANS physiological parameters, as well as in differential responses of Cases and Controls to ANS stressors.

Study 2 was designed to test the overall hypothesis that individuals with hyperresponsive ANS activity for developmental and/or genetic reasons are more likely to develop GWI when exposed to the physiological and psychological stresses of war together with wartime physical and chemical exposures, and that a specific genetic predisposing factor was BChE genotype. Performance of the autonomic stress tasks in Study 2 produced the expected physiological changes. Veterans who met rigorous criteria for GWI showed measurable, objective differences in a number of autonomic endpoints when compared to deployed and nondeployed Control groups. Some of the observed differences were evident in baseline measures (e.g., mean BP) that indicate greater sympathetic activity in Cases. The majority of differences, however, appeared in response to the stressors included in the test battery. The most notable changes were decreases in autonomic reactivity as measured by HRV during upright tilt and recovery, supporting our hypothesis that differences in reactivity discriminate between Case and Control groups.

The presence of a number of endpoints and a complex battery of tests in Study 2 raises the concern that our findings could be spurious. We do not believe this to be the Case. The pattern of significantly different endpoints between Cases and Controls is not random, nor is the direction of the observed differences. In all instances where differences were found in HRV measures, Cases were uniformly lower than Controls, suggesting a consistently lower level of autonomic responsiveness to stressors. When mean BP differences were found, Cases always had a higher mean BP than Controls. Other aspects of the study also give confidence in the findings. The number of participants was relatively

large for a physiologically-based study. Case and DC groups were selected to minimize differences in factors previously shown to be differentially associated with GWI risk.

Our results also underscore the importance of the use of a comprehensive test battery when assessing ANS function. If we had limited our task selection to just the Valsalva maneuver, as others have, we too would have concluded that no objective differences exist in ANS function between Cases and Controls. Our choice of study participants, however, potentially limits the generalizability of our results to other forms of GWI that may be more prevalent in veterans who served in other areas of the Gulf or who served in other branches of the military.

In light of investigations by other research groups during the data collection phases of the study, we requested an expansion of the original scope of work to include further statistical analysis of the epidemiological and exposure data obtained, to evaluate whether PON1 genotype and phenotype (enzyme velocity with three substrates) were associated with Case/Control status, and to further delineate the contribution of sympathetic output to the physiological differences observed between Cases and Controls.

To assess the validity of the self-reported exposures, we evaluated whether they "made sense" in the context of known factors about the Gulf War. Overall, the self-reported exposures were reasonable, providing an indication that further analyses relevant to exposures could be useful. In Study 1, the exposure associated with the highest risk of GWI was the use of PB. In the initial analysis of Study 2, only smoking during deployment was identified as a significant risk factor. But when duration of exposure was taken into account in supplementary analyses, exposure to pesticides and use of PB were also identified as risk factors. It is not obvious why smoking during deployment to the Persian Gulf 14 years previously was a significant risk factor in Study 2, but not in Study 1.

Variant volunteers were at increased risk for developing GWI if they reported use of PB during deployment. This finding is consistent with the expected greater sensitivity of carriers of low-velocity BChE mutations to PB. Our results, however, are based on a relatively small variant sample size, and must be interpreted with caution. Still, they provide a testable hypothesis that, if verified in future studies, may represent an important step forward in understanding possible associations of wartime exposures and the development of chronic health problems.

In summary, the results of the present studies indicate that in our samples of Gulf War Veterans, GWI was associated with: (1) altered autonomic function, (2) exposure to PB, and (3) being carriers of low-velocity mutations of BChE when combined together with exposure to PB. This last interaction produced the largest statistically significant risk, which remained highly elevated and significant when analyses were recomputed using the more lax CDC Case definition rather than the more restrictive KVP Case definition. Involvement of the ANS in GWI has also been studied recently by other investigators. Due to the many differences in terms of Case definition, Control selection and test protocols, results from other studies cannot be directly compared with ours. More extensive work is necessary to elucidate the mechanisms by which as-yet unidentified factors can lead to differences that persist over a decade after the war. Overall, our results, and those of other investigators indicate that further objective examination of autonomic function, and the ways in which wartime exposures and individual genetic variation interact in well-defined groups of veterans is warranted.

Section 6.
Table of Acronyms

AAS/AAN	American Autonomic Society/American Academy of Neurology
ABS HF	HRV absolute power in the high frequency band (0.15 Hz to 0.4 Hz.)
ABS LF	HRV absolute power in the low frequency band (0.04 Hz to < 0.15 Hz)
AChE	Acetylcholinesterase [EC 3.1.1.7]
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ARB	Autonomic reactivity battery
BChE	Butyrylcholinesterase [EC 3.1.1.8]
BP	Blood pressure
CARC	Chemical Agent Resistance Coating
CATI	Computer-assisted telephone interview
CDC	Centers for Disease Control
DBP	Diastolic blood pressure
DC	Deployed control group
ECG	Electrocardiogram
EMG	Electromyogram
GWI	Gulf War illnesses
HR	Heart rate
HRV	Heart rate variability
HSRRB	Surgeon General of the Army Human Subjects Research Review Board
IBI	Inter-beat interval

IRB	Institutional Review Board
KVP	Kansas Veterans' Project
NDC	Nondeployed control group
PB	Pyridostigmine bromide
PON1	Serum Paraoxonase [EC 3.1.8.1]
POWER	HRV spectral total power (0.003 Hz to 0.4 Hz)
PPI	Pre-pulse inhibition
PTSD	Post-traumatic stress disorder
PVC	Preventricular contraction
rMSSD	Root mean square of successive differences of adjacent normal R-R intervals
SBP	Systolic blood pressure
SDSD	Standard deviation of successive differences between adjoining normal R waves
SDNN	Standard deviation of all normal R-R intervals in each test
VARIANT	Individual who is a carrier of any of the low-velocity mutations of BChE, in this study any genotype other that U/U or U/K
%HF	HRV percent total power in the HF band (0.15 Hz to 0.4 Hz.)
%LF	HRV percent total power in the LF band (0.04 Hz to < 0.15 Hz)

Section 7.
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